Inter Partes Reexamination No. 95/001,096
Declaration of Campbell Rogers, M.D.
Exhibit 86

Guidant Press Releases > 2003

Jan 2, 2003

Guidant Reports Preliminary Results of DELIVER Clinical Trial

Conditions to Closing Guidant-Cook Merger Not Satisfied Company to Host Webcast/Conference Call January 3 at 8:30 AM EST

Indianapolis, Ind. - Guidant Corporation (NYSE and PCX: GDT), a world leader in the treatment of cardiac and vascular disease, today reported preliminary results of the DELIVER clinical trial. The DELIVER clinical trial was a randomized U.S. clinical study comparing the paclitaxel-coated ACHIEVE(tm) Drug Eluting Coronary Stent System, manufactured by Cook Incorporated, to the MULTI-LINK PENTA(tm) Coronary Stent System, manufactured by Guidant. The study was designed to demonstrate a 40 percent reduction in the primary endpoint of 270-day target vessel failure (TVF) for the ACHIEVE Drug Eluting Coronary Stent System, as compared to the PENTA Coronary Stent System.

While the final analysis of the DELIVER clinical results is still in progress, the preliminary analysis indicates that although there is a trend toward improvement in TVF, the primary endpoint will not be met. Additionally, while there appears to be a trend toward a reduced angiographic binary restenosis rate (ABRR), the planned 50 percent reduction in angiographic binary restenosis also will not be achieved. The percent reduction in both TVF and ABRR is less than expected due to the combination of excellent results in the PENTA Coronary Stent System control arm (9-month TVF of 14-15 percent and in-segment ABRR of 21-22 percent) and a higher-than-expected 11-12 percent TVF and 16-17 percent in-segment ABRR in the ACHIEVE arm of the study.

Based on these results, the conditions outlined in the previously announced Guidant-Cook Group Incorporated merger agreement are not expected to be satisfied. The terms of the merger agreement include a break-up fee of $50 million and an amendment to an existing stent delivery system agreement.

"Guidant"s collaboration with Cook represented a unique opportunity for both companies to advance drug eluting stent technologies in the field of vascular intervention," said John M. Capek, Ph.D., president, Vascular Intervention, Guidant. "While the DELIVER study did not meet its primary endpoint, the results demonstrate the safety and a trend toward efficacy of the ACHIEVE Drug Eluting Coronary Stent System. In addition, the study further demonstrates the excellent clinical performance of the PENTA Coronary Stent System. We look forward to a continued business relationship with Cook as we work toward our mutual goal of advancing medical technology."
Guidant"s Everolimus Program on Schedule

Guidant continues to make significant progress in its internal everolimus program. "We are enthusiastic about our work with everolimus, which is on track with excellent pre-clinical results. We look forward to the first human implant of an everolimus eluting stent later this quarter under the Vision-E trial, a feasibility study for an everolimus eluting MULTI-LINK VISION(tm) Coronary Stent System utilizing our proprietary TRUE COAT(tm) polymer," continued Capek. "In addition, our agreement in principle announced today to acquire the assets of Biosensors" everolimus eluting stent program strengthens our internal everolimus efforts."

Guidant issued a press release today announcing an agreement in principle to acquire certain assets of Biosensors International"s everolimus eluting stent program. The agreement is expected to provide Guidant with an exclusive worldwide license to Biosensors" intellectual property in the field of everolimus eluting stents, and a nonexclusive license to Biosensors" drug and bioabsorbable polymer formulation technology for use with other drugs.
Webcast

Guidant will host a live webcast briefing tomorrow, January 3, at 8:30 AM EST to discuss these events and 2003 financial guidance. Guidant President and CEO Ronald W. Dollens and John M. Capek, Ph.D., president, Vascular Intervention, will host the briefing. The webcast will be accessible through Guidant"s website at www.guidant.com/webcast or at CCBN"s individual investor center at www.companyboardroom.com. The webcast will be archived on both websites for future on-demand replay.

Guidant Corporation pioneers lifesaving technology, giving an opportunity for better life today to millions of cardiac and vascular patients worldwide. The company, driven by a strong entrepreneurial culture of more than 10,000 employees, develops, manufactures and markets a broad array of products and services that enable less-invasive care for some of life"s most threatening medical conditions. For more information visit www.guidant.com.

NOTE TO MEDIA: For more information about Guidant, including its products and services, please visit the company"s newsroom at www.guidant.com/newsroom.

This release includes forward-looking statements concerning anticipated clinical results, the company"s relationship with Cook, and the company"s everolimus program. The statements are based on assumptions about many important factors, including final adjudication of clinical results, litigation, product development timelines, the closing of the Biosensors transaction (which remains subject, among other things, to further due diligence and completion of a definitive agreement), and other factors identified on Exhibit 99.1 to the company"s most recent 10-Q. Actual results may differ materially. The company does not undertake to update its forward-looking statements.

System requirements for the webcast include Internet Explorer 5.0 (or higher) or Netscape Navigator 4.0 (or higher). Users also should have the most recent version of Windows Media Player, which can be downloaded for free at http://www.microsoft.com/windows/windowsmedia/en/download/. Users may experience varying levels of performance based on their connection speed, system capabilities and presence of a corporate firewall. To ensure a connection, users should go to the program five to 15 minutes before its start.

Guidant Press Releases > 2003

Jan 2, 2003

Guidant Reports Preliminary Results of DELIVER Clinical Trial

Conditions to Closing Guidant-Cook Merger Not Satisfied Company to Host Webcast/Conference Call January 3 at 8:30 AM EST

Indianapolis, Ind. - Guidant Corporation (NYSE and PCX: GDT), a world leader in the treatment of cardiac and vascular disease, today reported preliminary results of the DELIVER clinical trial. The DELIVER clinical trial was a randomized U.S. clinical study comparing the paclitaxel-coated ACHIEVE(tm) Drug Eluting Coronary Stent System, manufactured by Cook Incorporated, to the MULTI-LINK PENTA(tm) Coronary Stent System, manufactured by Guidant. The study was designed to demonstrate a 40 percent reduction in the primary endpoint of 270-day target vessel failure (TVF) for the ACHIEVE Drug Eluting Coronary Stent System, as compared to the PENTA Coronary Stent System.

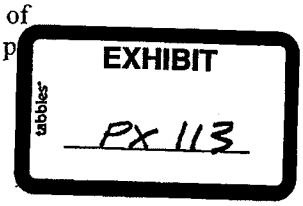
While the final analysis of the DELIVER clinical results is still in progress, the preliminary analysis indicates that although there is a trend toward improvement in TVF, the primary endpoint will not be met. Additionally, while there appears to be a trend toward a reduced angiographic binary restenosis rate (ABRR), the planned 50 percent reduction in angiographic binary restenosis also will not be achieved. The percent reduction in both TVF and ABRR is less than expected due to the combination of excellent results in the PENTA Coronary Stent System control arm (9-month TVF of 14-15 percent and in-segment ABRR of 21-22 percent) and a higher-than-expected 11-12 percent TVF and 16-17 percent in-segment ABRR in the ACHIEVE arm of the study.

Based on these results, the conditions outlined in the previously announced Guidant-Cook Group Incorporated merger agreement are not expected to be satisfied. The terms of the merger agreement include a break-up fee of $50 million and an amendment to an existing stent delivery system agreement.

"Guidant"s collaboration with Cook represented a unique opportunity for both companies to advance drug eluting stent technologies in the field of vascular intervention," said John M. Capek, Ph.D., president, Vascular Intervention, Guidant. "While the DELIVER study did not meet its primary endpoint, the results demonstrate the safety and a trend toward efficacy of the ACHIEVE Drug Eluting Coronary Stent System. In addition, the study further demonstrates the excellent clinical performance of the PENTA Coronary Stent System. We look forward to a continued business relationship with Cook as we work toward our mutual goal of advancing medical technology."
Guidant"s Everolimus Program on Schedule

Guidant continues to make significant progress in its internal everolimus program. "We are enthusiastic about our work with everolimus, which is on track with excellent pre-clinical results. We look forward to the first human implant of an everolimus eluting stent later this quarter under the Vision-E trial, a feasibility study for an everolimus eluting MULTI-LINK VISION(tm) Coronary Stent System utilizing our proprietary TRUE COAT(tm) polymer," continued Capek. "In addition, our agreement in principle announced today to acquire the assets of Biosensors" everolimus eluting stent program strengthens our internal everolimus efforts."

Guidant issued a press release today announcing an agreement in principle to acquire certain assets of Biosensors International"s everolimus eluting stent program. The agreement is expected to provide Guidant with an exclusive worldwide license to Biosensors" intellectual property in the field of everolimus eluting stents, and a nonexclusive license to Biosensors" drug and bioabsorbable p



formulation technology for use with other drugs.
Webcast

Guidant will host a live webcast briefing tomorrow, January 3, at 8:30 AM EST to discuss these events and 2003 financial guidance. Guidant President and CEO Ronald W. Dollens and John M. Capek, Ph.D., president, Vascular Intervention, will host the briefing. The webcast will be accessible through Guidant"s website at www.guidant.com/webcast or at CCBN's individual investor center at www.companyboardroom.com. The webcast will be archived on both websites for future on-demand replay.

Guidant Corporation pioneers lifesaving technology, giving an opportunity for better life today to millions of cardiac and vascular patients worldwide. The company, driven by a strong entrepreneurial culture of more than 10,000 employees, develops, manufactures and markets a broad array of products and services that enable less-invasive care for some of life"s most threatening medical conditions. For more information visit www.guidant.com.

NOTE TO MEDIA: For more information about Guidant, including its products and services, please visit the company"s newsroom at www.guidant.com/newsroom.

This release includes forward-looking statements concerning anticipated clinical results, the company"s relationship with Cook, and the company"s everolimus program. The statements are based on assumptions about many important factors, including final adjudication of clinical results, litigation, product development timelines, the closing of the Biosensors transaction (which remains subject, among other things, to further due diligence and completion of a definitive agreement), and other factors identified on Exhibit 99.1 to the company"s most recent 10-Q. Actual results may differ materially. The company does not undertake to update its forward-looking statements.

System requirements for the webcast include Internet Explorer 5.0 (or higher) or Netscape Navigator 4.0 (or higher). Users also should have the most recent version of Windows Media Player, which can be downloaded for free at http://www.microsoft.com/windows/windowsmedia/en/download/. Users may experience varying levels of performance based on their connection speed, system capabilities and presence of a corporate firewall. To ensure a connection, users should go to the program five to 15 minutes before its start.

The American Journal of Cardiology

AUGUST 15, 1998

Visit our INTERNET Home Page:
http://www.elsevier.com/locate/amjcard

EXCERPTA MEDICA

coronary ultrasound.[13,14] The current study extends the concept that coronary angiography and examination of coronary calcium provide information on different aspects of coronary atherosclerotic disease (i.e., stenosis severity caused by atherosclerotic plaque as opposed to atherosclerotic plaque itself). The indirect relations between these 2 aspects explain why there was only a moderate site-by-site correlation (i.e., predictive accuracy of spotty coronary calcium). Future coronary calcium studies with angiographic and intracoronary ultrasound correlates may clarify why the formation of calcified plaques leads to luminal narrowing in some cases but not in others.

The results of this study indicate that in patients with normal or near-normal angiograms, spotty coronary calcium is associated with slight angiographic changes consistent with early atherosclerotic disease and compensatory arterial remodeling. This confirms the concept that coronary angiography and examination of coronary calcium provide information on different aspects of coronary atherosclerotic disease (i.e., stenosis severity caused by atherosclerotic plaque as opposed to atherosclerotic plaque itself).

1. Rumberger JA, Sheedy PF, Breen JR, Schwartz RS. Coronary calcium as determined by electron beam computed tomography, and coronary disease on arteriogram: effect of patient's sex on diagnosis. *Circulation* 1995;91:1363–1367.
2. Budoff, MJ, Georgiou D, Brody A, Agatston AS, Kennedy J, Wolfkiel C, Stanford W, Shields P, Lewis RJ, Janowitz WR, Rich S, Brundage BH. Ultrafast computed tomography as a diagnostic modality in the detection of coronary artery disease. A multicenter study. *Circulation* 1996;93:898–904.
3. Rumberger JA, Sheedy PF, Breen JF, Schwartz RS. Electron beam computed tomographic calcium score cutpoints and severity of associated angiographic lumen stenosis. *J Am Coll Cardiol* 1997;29:1542–1548.
4. Kajinami K, Seki H, Takekoshi N, Mabuchi H. Coronary calcification and coronary atherosclerosis: site by site comparative morphologic study of electron beam computed tomography and coronary angiography. *J Am Coll Cardiol* 1997;29:1549–1556.
5. Sangiorgi G, Rumberger J, Severson A, Edwards WD, Gregoire J, Fitzpatrick LA, Schwartz RS. Arterial calcification and not lumen stenosis is correlated with atherosclerotic plaque burden in humans: a histologic study of 723 coronary artery segments using nondecalcifying methodology. *J Am Coll Cardiol* 1998; 31:126–133.
6. Kaufmann RB, Sheedy PF, Maher JE, Bielak LF, Breen JF, Schwartz RS, Peyser PA. Quantity of coronary artery calcium detected by electron beam computed tomography in asymptomatic subjects and angiographically studied patients. *Mayo Clin Proc* 1995;70:223–232.
7. Dodge JTJr, Brown G, Bolson EL, Dodge HT. Lumen diameter of normal human coronary arteries: influence of age, sex, anatomic variation, and left ventricular hypertrophy or dilation. *Circulation* 1992;86:232–246.
8. Bell MR, Britson PJ, Chu A, Holmes DR Jr, Bresnahan JF, Schwartz RS. Validation of a new UNIX-based quantitative coronary angiographic system for the measurement of coronary artery lesions. *Cathet Cardiovasc Diagn* 1997;40: 66–74.
9. American Heart Association Committee Report. A reporting system on patients evaluated for coronary artery disease. *Circulation* 1975;51:7–34.
10. Schmermund A, Rensing BJ, Sheedy PF, Bell MR, Rumberger JA. Intravenous electron-beam CT coronary angiography for segmental analysis of coronary artery stenoses. *J Am Coll Cardiol* 1998;31:1547–1554.
11. de Feyter PJ, Serruys PW, Davies MJ, Richardson P, Lubsen J, Oliver MF. Quantitative coronary angiography to measure progression and regression of coronary atherosclerosis. Value, limitations, and implications for clinical trials. *Circulation* 1991;84:412–423
12. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol* 1990;15:827–832.
13. Baumgart D, Schmermund A, Görge G, Haude M, Ge J, Adamzik M, Schnert C, Altmaier K, Grönemeyer D, Seibel R, Erbel R. Comparison of electron beam computed tomography with intracoronary ultrasound and coronary angiography for the detection of coronary atherosclerosis. *J Am Coll Cardiol* 1997;30:57–64.
14. Schmermund A, Baumgart D, Adamzik M, Ge J, Grönemeyer D, Seibel R, Schnert C, Görge G, Haude M, Erbel R. Comparison of electron-beam computed tomography and intracoronary ultrasound in detecting calcified and non-calcified plaques in patients with acute coronary syndromes and no or minimal to moderate angiographic coronary artery disease. *Am J Cardiol* 1998;81:141–146.

Effect of Direct Thrombin Inhibition With *Bivalirudin (Hirulog)* on Restenosis After Coronary Angioplasty*

J.E.B. Burchenal, MD, David S. Marks, MD, J. Tift Mann, MD, Marc J. Schweiger, MD, Martin T. Rothman, MD, Peter Ganz, MD, Burt Adelman, MD, and John A. Bittl, MD

Percutaneous transluminal coronary angioplasty is effective but its long-term efficacy is limited by restenosis.[1] Multiple attempts to abrogate this process pharmacologically have been unsuccessful.[2] Restenosis is a complex process involving many redundant pathways including smooth muscle cell proliferation, inflammation, thrombosis, and vascular remodeling. Thrombin could contribute to this process by promoting smooth muscle cell proliferation,[3] growth factor and cytokine release,[4,5] platelet activation,[6] and the formation of fibrin.[7] Therefore, by modulating these processes, inhibition of thrombin could reduce restenosis. Thrombin inhibition by heparin is limited by its inability to inhibit fibrin-bound thrombin, the need for a cofactor, and its inactivation by plasma inhibitors. Bivalirudin (Hirulog [Biogen, Cambridge, Massachusetts]) is derived from the medicinal leech, *Hirudo medicinalis*,[8] and is a potent direct inhibitor of thrombin activity. A recent pilot study found bivalirudin to be a safe adjunctive therapy during coronary angioplasty.[9] Animal studies have suggested that direct thrombin inhibitors can reduce restenosis.[10] Because of these encouraging animal results and the established clinical safety of bivalirudin, a trial with angiographic follow-up in humans was performed nested within the multicenter Hirulog Angioplasty Study to

From the Departments of Medicine, Brigham and Women's Hospital, Boston, Massachusetts; Wake Heart Associates, Raleigh, North Carolina; Baystate Medical Center, Springfield, Massachusetts; London Chest Hospital, London, United Kingdom; and Biogen, Inc., Cambridge, Massachusetts. This study was supported in part by a research grant from Biogen, Inc., Cambridge, Massachusetts. Dr. Burchenal's address is: Division of Cardiology, Campus Box 132, University of Colorado Health Sciences Center, 4200 East Ninth Avenue, Denver, Colorado 80262. Manuscript received December 8, 1997; revised manuscript received and accepted March 18, 1998.

*Presented in part at the 68th Scientific Sessions of the American Heart Association, November 13–16, 1995.

0002-9149/98/$19.00 511
PII S0002-9149(98)00371-3

TABLE 1 Baseline Characteristics and Parameters of Restenosis

	Bivalirudin (n = 37)	Heparin (n = 50)	p Value
Clinical Characteristics			
Age (yr)	65 ± 11	60 ± 11	0.04
Sex (% women)	32.4	30.0	0.81
Diabetes (%)	24.3	22.0	0.80
Tobacco use (%)	27.0	20.0	0.44
Previous PTCA (%)	13.5	20.0	0.43
Postinfarction angina (%)	32.4	28.0	0.66
Rest pain (%)	70.3	72.0	0.86
Follow-up time (mo)	4.5 ± 2.4	4.3 ± 1.8	0.73
Angiographic characteristics			
No. of lesions	43	55	
LAD location (%)	27.9	30.9	0.75
Total occlusion (%)	2.3	9.1	0.23
Reference diameter (mm)	2.60 ± 0.58	2.28 ± 0.46	0.27
Preprocedure minimal lumen diameter (mm)	0.68 ± 0.35	0.55 ± 0.32	0.05
Preprocedure diameter stenosis (%)	71.7 ± 11.5	75.5 ± 12.6	0.12
Postprocedure minimal lumen diameter (mm)	1.50 ± 0.43	1.38 ± 0.31	0.14
Postprocedure diameter stenosis (%)	36.6 ± 10.1	38.3 ± 9.8	0.41
Acute gain (mm)	0.82 ± 0.37	0.84 ± 0.38	0.85
Angiographic parameters of restenosis			
Restenosis rate (no. (%))	23 (62.2)	29 (58.0)	0.70
Follow-up diameter stenosis (%)	55.8 ± 17.8	57.1 ± 22.8	0.75
Follow-up minimal lumen diameter (mm)	1.06 ± 0.51	1.00 ± 0.61	0.60
late loss (mm)	0.44 ± 0.47	0.39 ± 0.53	0.62
late loss index	0.49 ± 0.62	0.48 ± 0.74	0.90
Clinical events at 6 mo			
Any clinical event (no. (%))	14 (37.8)	18 (36.7)	0.92
Death (no. (%))	0	0	1.00
Myocardial infarction (no. (%))	4 (10.8)	0	0.03
Revascularization (no. (%))	12 (32.4)	18 (36.7)	0.68

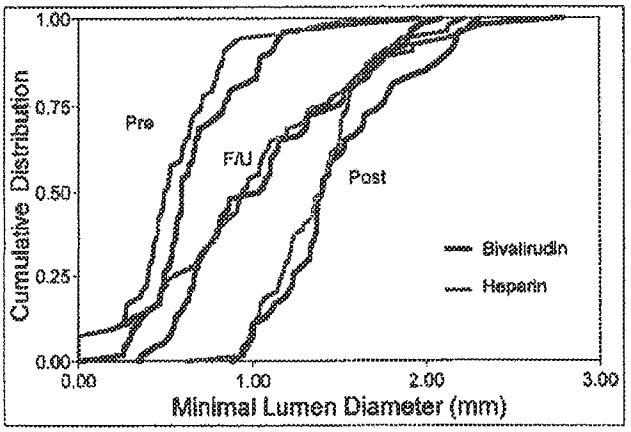


FIGURE 1. Cumulative distribution of values for minimal lumen diameter in the heparin and bivalirudin groups before (Pre) and after (Post) angioplasty and at the time of follow-up angiography (F/U). The distributions at these time points were not significantly different (Pre, p = 0.054; Post, p = 0.14; F/U, p = 0.60), and neither the acute gain (Post-Pre, p = 0.85) nor the late loss (Post-F/U, p = 0.62) were significantly different.

determine if direct thrombin inhibition with bivalirudin could inhibit restenosis.

* * *

Between July 22, 1993, and July 15, 1994, 4,098 patients were entered into the Hirulog Angioplasty Study and randomized to treatment with bivalirudin or heparin during percutaneous transluminal coronary angioplasty for unstable angina. From this group, 244 consecutive patients at 15 sites were enrolled in an angiographic follow-up substudy. All patients gave informed consent to the protocol, which was approved by the Institutional Review Board of each site. All patients had successful angioplasty, defined as <50% residual stenosis without in-hospital complication (death, myocardial infarction, bypass surgery, or repeat angioplasty). Follow-up for this study was prematurely terminated when the results of the parent trial were released. At the time of study termination, 6-month angiographic follow-up had been obtained in 87 patients. The complete protocol of the Hirulog Angioplasty Study has been detailed previously.[13] Briefly, immediately before angioplasty, patients were randomly assigned to therapy with either bivalirudin (bolus 1.0 mg/kg, 4-hour infusion at 2.5 mg/kg/hour, 14- to 20-hour infusion at 0.2 mg/kg/hour) or heparin (bolus 175 U/kg, 18- to 24-hour infusion at 15 U/kg/hour). Activated clotting time (ACT) was measured in both study groups 5 and 45 minutes after administration of the bolus dose. If the ACT was <350 seconds, patients given heparin were treated with a heparin bolus of 60 U/kg, and bivalirudin patients were treated with a saline bolus. Patients were asked to return for follow-up angiography 6 months after the procedure, and clinical follow-up was also obtained in all patients at 6 months.

Quantitative coronary angiographic analysis (ImageComm Systems, Inc., Sunnyvale, California)[12] was used to determine stenosis severity before and after angioplasty, and at follow-up. Acute gain was defined as the increase in minimal lumen diameter (MLD) produced by the angioplasty. Late loss was defined as the subsequent reduction in MLD between the time of intervention and follow-up angiography. Restenosis was defined primarily as the dichotomous outcome of ≥50% diameter stenosis at follow-up, and secondarily as the continuous variables of late loss, MLD at follow-up, and percent diameter stenosis at follow-up.

Statistical analysis was performed using likelihood

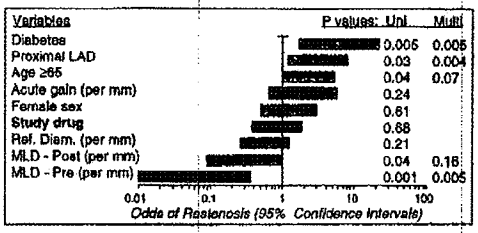


FIGURE 2. Predictors of restenosis. Odds ratios (and 95% confidence intervals) are provided to estimate the probability that a given variable increases or decreases the likelihood of restenosis compared with all other lesions without the variable. For continuous variables, such as acute gain, reference vessel diameter (Ref. Diam.), preprocedure minimal lumen diameter (MLD-Pre), and postprocedure minimal lumen diameter (MLD-Post), the odds ratio refers to the added or reduced risk of one additional mm in vessel size. The p values are obtained from univariate (Uni) and multivariate (Multi) logistic regression analyses of restenosis, as defined dichotomously as the presence of ≥50.0% stenosis on follow-up angiography.

TABLE II Risk Factors for Restenosis

	Restenosis	No Restenosis	Univariate p Value	Multivariate p Value
Clinical risk factors				
Age >65 (%)	53.9	31.4	0.04	0.07
Female gender (%)	32.7	28.6	0.61	
Diabetes (%)	32.7	8.6	0.005	0.005
Previous PTCA (%)	13.5	22.9	0.26	
Rest pain (%)	65.4	80.0	0.16	
Postinfarction angina (%)	25.0	37.1	0.23	
Tobacco use (%)	21.2	25.7	0.62	
Angiographic risk factors				
LAD location (%)	38.6	17.1	0.03	0.004
Total occlusion (%)	7.0	4.9	1.00	
Reference diameter (mm)	2.27 ± 0.42	2.41 ± 0.63	0.21	
Preprocedure minimal lumen diameter (mm)	0.50 ± 0.24	0.75 ± 0.41	0.001	0.005
Postprocedure minimal lumen diameter (mm)	1.37 ± 0.32	1.53 ± 0.42	0.04	0.16
Acute gain (mm)	0.87 ± 0.36	0.78 ± 0.39	0.24	

ratio chi-square and Fisher's exact tests for categorical variables, and unpaired *t* tests for continuous variables. Linear and logistic regression were used to identify predictors of restenosis. Power calculations were performed to determine the overall power of the study and to determine the magnitude of the differences in variables that could have been detected by this study given the actual sample size.[13] All statistical analyses were completed with standard statistical software (SYSTAT 5.2, Evanston, Illinois). All values are presented as mean ± SD.

Angiographic follow-up was obtained in 87 patients 4.4 ± 2.1 months after angioplasty. Patients who received bivalirudin were slightly older than those who received heparin (65 ± 11 vs 60 ± 11 years, p = 0.04); otherwise, there were no significant differences in clinical characteristics between treatment groups. The distribution of these clinical characteristics was also similar to that seen in the Hirulog Angioplasty Study.[11] In these 87 patients, 98 lesions were dilated. The angiographic characteristics of these lesions also did not differ significantly between treatment groups (Table I).

The primary end point of restenosis defined as ≥50% diameter stenosis at follow-up occurred in 23 of 37 patients treated with bivalirudin (62.2%) and in 29 of 50 patients treated with heparin (58.0%, p = 0.70). Similarly, restenosis occurred in 26 of 43 lesions treated with bivalirudin (60.5%) and in 31 of 55 lesions treated with heparin (56.4%, p = 0.68). There was also no significant difference in restenosis between treatment groups using the secondary measures of late loss, MLD at follow-up, and percent diameter stenosis at follow-up (Table I and Figure 1). The ratio of late loss to acute gain (the loss index) was not significantly affected by treatment with bivalirudin (0.49 ± 0.62) or heparin (0.48 ± 0.74, p = 0.90). The incidence of death or repeat revascularization at 6 months was similar between the 2 groups. There were more myocardial infarctions at 6 months among bivalirudin patients, but the number of events was small. The overall incidence of any clinical events at 6 months was not significantly different between bivalirudin (37.8%) and the heparin (36.7%, p = 0.92) patients (Table I).

Logistic regression analysis was used to identify predictors of restenosis (Figure 2). On univariate analysis, restenosis was related to age (>65 years) (odds ratio [OR] of restenosis 2.39, 95% confidence interval [CI] 1.04 to 5.54; p = 0.04), diabetes (OR 6.33, CI 1.73 to 23.19; p = 0.005), lesion location in the proximal left anterior descending artery (OR 3.05, CI 1.15 to 8.08, p = 0.03), to the preprocedure MLD (OR 0.07, CI 0.02 to 0.37 per additional mm increase; p <0.001), and to the postprocedure MLD (OR 0.30, CI 0.09, 0.96 per additional mm increase; p = 0.04). On multivariate analysis, however, only diabetes (p = 0.005), lesion location in the left anterior descending artery (p = 0.004) and preprocedure MLD (p = 0.005) emerged as independent predictors of restenosis (Table II). Linear regression analysis was used to identify predictors of late loss. On univariate analysis, late loss was directly related to postprocedural MLD (p = 0.06), preprocedural MLD (p = 0.01), and acute gain (Figure 3, p <0.001). In the multivariate model, only acute gain (p <0.001) emerged as a significant predictor of late loss.

This study had sufficient power (80%) to detect a 68% decrease in late loss. Similarly, this study had a 54% chance (power of 54%) of detecting a 50% reduction in the restenosis rate as measured by late loss.

• • •

Direct thrombin inhibition with bivalirudin does not inhibit restenosis after coronary angioplasty. Re-

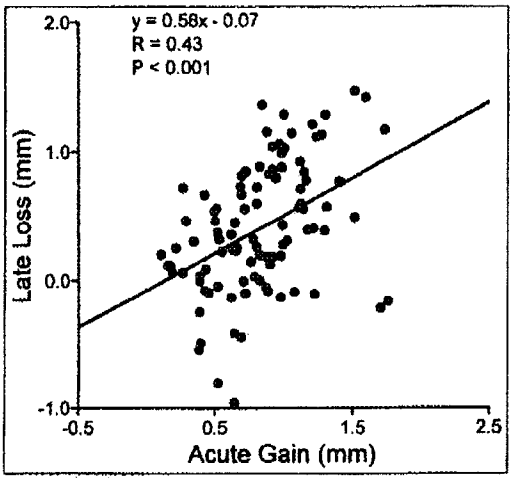


FIGURE 3. Scatter plot with linear regression analysis of the significant relation between acute gain (postprocedure MLD, preprocedure MLD) and late loss (postprocedure MLD, follow-up MLD). Acute gain was the only significant and independent predictor of late loss [R = 0.43, p <0.001] among angiographic parameters measured.

stenosis, whether defined as the dichotomous variable of ≥50% diameter stenosis or as the continuous variables of percent diameter stenosis and MLD at follow-up angiography, was not significantly reduced by treatment with bivalirudin. The bivalirudin and heparin patient populations were similar, indicating adequate randomization, and the 2 groups had similar risk factors for restenosis. Multivariate analysis revealed clinical and angiographic predictors of restenosis that were consistent with previous studies.[2] Bivalirudin treatment did not significantly alter the impact of these predictors on restenosis. Similarly, treatment with bivalirudin did not affect the ratio of late loss to acute gain (the loss index). This lack of effect on risk factors and the loss index implies that antithrombin therapy did not significantly modify the basic pathophysiology of the restenotic process. Therefore, direct thrombin inhibition for 24 hours after angioplasty is not sufficient to significantly alter restenosis.

Two possible explanations for this negative finding are that thrombin activity may not be of primary importance to the restenotic process, or that the treatment may not have been of sufficient length. Restenosis is a complex, multifactorial process. Studies have demonstrated the importance of vascular remodeling in restenosis,[14] and, because thrombin is unlikely to make a significant contribution to remodeling, thrombin inhibition with bivalirudin would not be expected to decrease remodeling. This lack of impact on remodeling may explain bivalirudin's lack of effect on restenosis. Although there is evidence that supports the role for thrombin in restenosis from in vitro,[7] animal,[10,15] and clinical studies,[16] previous clinical trials of thrombin inhibitors in restenosis have come to the same conclusions as this present study and support the hypothesis that inhibition of thrombin alone is not sufficient to alter the restenotic process.[17,18]

The second major possible explanation for this study's results is the duration of treatment. Although most platelet activation and thrombosis occurs in the first 24 to 48 hours after angioplasty, some thrombin generation appears to continue until the angioplasty site is completely reendothelialized.[19] Therefore, it is possible that treatment for only 24 hours after angioplasty may not have been sufficient to passivate the arterial wall and reduce restenosis.

The sample size of this study only allows us to be confident of these null results with a power of 54%. Because there are no trends in the data in favor of treatment, it is unlikely that a clinically significant difference would have been found even with greater subject numbers.

In conclusion, direct thrombin inhibition with bivalirudin does not significantly affect restenosis after angioplasty. Bivalirudin also does not alter the impact of established risk factors on restenosis.

1. Holmes DR Jr, Vlietstra RE, Smith HC, Vetrovec GW, Kent KM, Cowley MJ, Faxon DP, Gruentzig AR, Kelsey SF, Detre KM, van Raden MJ, Mock MB. Restenosis after percutaneous transluminal coronary angioplasty (PTCA): a report from the PTCA Registry of the National Heart, Lung, and Blood Institute. *Am J Cardiol* 1984;53:77C–81C.
2. Hillegass WB, Ohman EM, Califf RM. Restenosis: the clinical issues. In: Topol EJ, ed. Textbook of Interventional Cardiology Philadelphia: WB Saunders, 1994:415–435.
3. McNamara CA, Sarembock IJ, Gimple LW, Fenton JW II, Coughlin SR, Owens GK. Thrombin stimulates proliferation of cultured rat aortic smooth muscle cells by a proteolytically activated receptor. *J Clin Invest* 1993;91:94–98.
4. Harlan JM, Thompson PJ, Ross RR, Bowen-Pope DF. Alpha-thrombin induces release of platelet-derived growth factor-like molecule(s) by cultured human endothelial cells. *J Cell Biol* 1986;103:1129–1133.
5. Jones A, Geczy CL. Thrombin and factor Xa enhance the production of interleukin-1. *Immunology* 1990;71:236–241.
6. Eidt JF, Allison P, Noble S, Ashton J, Golino P, McNatt J, Buja LM, Willerson JT. Thrombin is an important mediator of platelet aggregation in stenosed canine coronary arteries with endothelial injury. *J Clin Invest* 1989;84:18–27.
7. Wilcox JN, Thrombin and other potential mechanisms underlying restenosis. *Circulation* 1991;84:432–435.
8. Maraganore JM, Bourdon P, Jablonski J, Ramachandran KL, Fenton JW II. Design and characterization of hirulogs: a novel class of bivalent peptide inhibitors of thrombin. *Biochemistry* 1990;29:7095–7101.
9. Topol EJ, Bonan R, Jewitt D, Sigwart U, Kakkar VV, Rothman M, de Bono D, Ferguson J, Willerson JT, Strony J, Ganz P, Cohen MD, Raymond R, Fox I, Maraganore J, Adelman B. Use of a direct antithrombin, hirulog, in place of heparin during coronary angioplasty. *Circulation* 1993;87:1622–1629.
10. Sarembock IJ, Gertz SD, Gimple LW, Owen RM, Powers ER, Roberts WC. Effectiveness of recombinant desulfatohirudin in reducing restenosis after balloon angioplasty of atherosclerotic femoral arteries in rabbits. *Circulation* 1991; 84:232–243.
11. Bittl JA, Strony J, Brinker JA, Ahmed WH, Meckel CR, Chaitman BR, Maraganore J, Deutsch E, Adelman B, for the Hirulog Angioplasty Study Investigators. Treatment with bivalirudin (Hirulog) as compared with heparin during coronary angioplasty for unstable or postinfarction angina. *N Engl J Med* 1995; 333:764–769.
12. Mancini GB, Simon SB, McGillem MJ, LeFree MT, Friedman HZ, Vogel RA. Automated quantitative coronary arteriography: morphologic and physiologic validation in vivo of a rapid digital angiographic method. *Circulation* 1987;75:452–460.
13. Rosner B. Estimation of sample size and power for comparing two means. In: Rosner B, ed. Fundamentals of Biostatistics. Boston: PWS-Kent Publishing, 1990:274–275.
14. Mintz GS, Popma JJ, Pichard AD, Kent KM, Satler LF, Wong SC, Hong MK, Kovach JA, Leon MB. Arterial remodeling after coronary angioplasty: a serial intravascular ultrasound study. *Circulation* 1996;94:35–43.
15. Unterberg C, Sandrock D, Nebendahl K, Buchwald AB. Reduced acute thrombus formation results in decreased neointimal proliferation after coronary angioplasty. *J Am Coll Cardiol* 1995;26:1747–1754.

16. Violaris AG, Melkert R, Herrman JR, Serruys PW. Role of angiographically identifiable thrombus on long-term luminal renarrowing after coronary angioplasty. A quantitative angiographic analysis. *Circulation* 1996;93:889–897.
17. Ellis SG, Roubin GS, Wilentz J, Douglas JS Jr, King SB III. Effect of 18- to 24-hour heparin administration for prevention of restenosis after uncomplicated coronary angioplasty. *Am Heart J* 1989;117:777–782.
18. Serruys PW, Herrman JP, Simon R, Rutsch W, Bode C, Laarman GJ, van Dijk R, van den Bos AA, Umans VA, Fox KA, Close P, Deckers JW, for the Helvetica Investigators. A comparison of hirudin with heparin in the prevention of restenosis after coronary angioplasty. *N Engl J Med* 1995;333:757–763.
19. Barry WL, Gimple LW, Humphries JE, Powers ER, McCoy KW, Sanders JM, Owens GK, Sarembock IJ. Arterial thrombin activity after angioplasty in an atherosclerotic rabbit model: time course and effect of hirudin. *Circulation* 1996;94:88–93.

Predictive Value of C-Reactive Protein After Successful Coronary-Artery Stenting in Patients With Stable Angina

Achille Gaspardone, MPhil, MD, Filippo Crea, MD, Francesco Versaci, MD, Fabrizio Tomai, MD, Antonio Pellegrino, MD, Luigi Chiariello, MD, and Pier A. Gioffrè, MD

Coronary artery stenting is now used in many laboratories as the primary therapy for coronary artery lesions in patients with ischemic heart disease. However, intracoronary stents are still plagued by a considerable risk of restenosis.[1] Serial intravascular ultrasound studies suggest that neointimal proliferation through the stent struts, rather than arterial remodeling, accounts for almost all the late loss in lumen diameter.[2] Recent reports indicate that inflammatory mechanisms play a crucial role in the pathogenesis of neointimal proliferation and, as a consequence, in the process of coronary restenosis.[3,4] In particular, interleukin-1 and 6, secreted by activated macrophages are powerful stimuli for smooth muscle cell proliferation and hepatocyte production of a series of acute-phase proteins such as C-reactive protein (CRP).[5,6] The latter is the prototypic acute-phase protein; its blood concentration increases from trace levels in healthy subjects to over a 1,000-fold within 24 to 48 hours after an inflammatory stimulus.[7] Thus, plasma levels of CRP after coronary artery stenting could be a marker of the intensity of the inflammatory reaction responsible for neointimal proliferation and subsequent restenosis. We tested this hypothesis in a group of patients with stable angina pectoris who underwent successful coronary artery stenting.

• • •

The study group consisted of 81 consecutive patients (mean age 59 ± 8 years; range 43 to 77) with the following characteristics: chronic stable angina pectoris, 1-vessel coronary artery disease (defined as a reduction ≥70% of the luminal diameter, as measured by quantitative computerized angiography, extending ≤15 mm in length in a vessel ≥3 mm in diameter) in the proximal third of 1 major epicardial coronary artery, left ventricular ejection fraction ≥45%, preprocedural CRP plasma levels within the normal limits, and elective and successful coronary artery stenting. Coronary lesions were classified according to the system of the American College of Cardiology–American Heart Association Task Force.[8] Procedural success was defined as residual stenosis <30% in the worse of 2 orthogonal views, as assessed by quantitative analysis, normal runoff of the contrast medium in the stented vessel, and absence of death, myocardial infarction, and the need for further revascularization procedures during the hospital stay.

Patients received aspirin, ticlopidine, and diltiazem 2 days before the procedure. Monorail balloon catheters were used in all patients to predilate the stenosis. Ioversol (Optiray 320, Mallinckrodt Medical, St. Louis, Missouri) was used as a contrast agent. Angioplasty was performed with the use of conventional techniques. All the stents (Johnson and Johnson Interventional Systems, Warren, New Jersey) were manually mounted on a balloon that matched the angiographic reference lumen diameter in all patients. All stents were overdilated at high pressure. A PS153 stent was used in 14 cases and a PS 154A in 67. Stents were implanted in 51 patients with stenosis of the left anterior descending coronary artery, in 20 patients with stenosis of right coronary artery, and in 10 patients with stenosis of the circumflex artery. After discharge, ticlopidine was continued for 1 month and aspirin and diltiazem indefinitely.

Venous blood samples were obtained on admission to the hospital and 6, 24, 48, and 72 hours after the procedure. Plasma samples were immediately analyzed for CRP concentration, which was immunologically determined by the immunoturbidimetric method (Roche Unimate 3 CRP, Milan, Italy). The normal upper reference value for CRP with this method is up to 0.5 mg/dl.[9] For total creatine kinase (CK), the MB isoform of creatine kinase (CK-MB), and cardiac troponin I, venous blood samples were obtained immediately before the procedure and 6, 24, and 48 hours after the procedure. Plasma samples were immediately analyzed for CK and CK-MB with commercially available immunochemical tests. Cardiac troponin I was immediately analyzed by immunoenzymatic "sandwich" assay (Access Cardiac Troponin I).

From the Divisione di Cardiochirurgia, Università di Roma Tor Vergata, Rome, Italy. This study was supported by a grant from the Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST-60% 1997), Rome, Italy. Dr. Gaspardone's address is: Divisione di Cardiochirurgia, Università di Roma Tor Vergata via Portuense 700, 00149 Rome, Italy. Manuscript received January 15, 1998; revised manuscript received and accepted March 25, 1998.

0002-9149/98/$19.00
PII S0002-9149(98)00370-1

Circulation

Volume 94, Number 7 October 1, 1996

Editorials

Brief Rapid Communication

Clinical Investigation and Reports

Basic Science Reports

Current Perspectives

James B. Herrick Lecture

Images in Cardiovascular Medicine

AHA Medical/Scientific Statement

73-3221(SP) ISSN 0009-7322

Fish Oils and Low-Molecular-Weight Heparin for the Reduction of Restenosis After Percutaneous Transluminal Coronary Angioplasty

The EMPAR Study

John A. Cairns, MD; John Gill, MD; Brian Morton, MD; Robin Roberts, MTech; Michael Gent, DSc; Jack Hirsh, MD; Douglas Holder, MD; Keith Finnie, MB,ChB; Jean François Marquis, MD; Salim Naqvi, MD; Eric Cohen, MD; for the EMPAR Collaborators

Background Percutaneous transluminal coronary angioplasty (PTCA) is complicated by restenosis within 6 months in >40% of patients. Theoretical, animal experimental, and human epidemiological and clinical trial findings have suggested that fish oils (n-3) might reduce restenosis. Low-molecular-weight heparin (LMWH) has reduced cellular proliferation and restenosis in several experimental systems.

Methods and Results We randomized 814 patients to fish oils (5.4 g n-3 fatty acids) or placebo a median of 6 days before PTCA and continued for 18 weeks. At the time of sheath removal, 653 patients with at least one successfully dilated lesion were randomized to LMWH (30 mg SC BID) or control for 6 weeks in a 2×2 factorial design. Follow-up with quantitative coronary angiography (QCA; target, 18 weeks) was interpretable on 96% of these patients. Restenosis rates per patient were for n-3, 46.5%; placebo, 44.7%; LMWH, 45.8%; and control, 45.4%. Restenosis rates per lesion were for n-3, 39.7%; placebo, 38.7%; LMWH, 38%; and control, 40.4%. At follow-up QCA, mean minimal lumen diameters were (mm) for n-3, 1.12; placebo, 1.10; LMWH, 1.12; and control, 1.10. Fifteen percent of patients permanently discontinued n-3/placebo before study completion, and 21% of patients discontinued LMWH early. There were no significant differences in the occurrences of ischemic events. Bleeding was more common with LMWH, usually was mild, and led to early discontinuation of study medication in only 0.9% of patients. Gastrointestinal side effects were more common in patients receiving n-3 than placebo.

Conclusions There is no evidence for a clinically important reduction of PTCA restenosis in this trial by either n-3 or LMWH. Evaluation of the results for n-3 in the context of previously published data on the reduction of PTCA restenosis indicates that n-3 is not efficacious and that further trials are unwarranted. (*Circulation*. 1996;94:1553-1560.)

Key Words • angioplasty • restenosis • fish oils • heparin • coronary disease

The incidence of restenosis after PTCA has decreased little since the earliest days of angioplasty and remains at >40% by 6 months.[1,2] More than half the affected patients have a recurrence of angina sufficient to warrant repeated PTCA or coronary artery bypass surgery.[2] No medical intervention has emerged as clearly efficacious in reducing the risk of restenosis.[3]

Coronary events may be less frequent among populations consuming large amounts of n-3 polyunsaturated fatty acids (fish oils),[4] and clinical trials among survivors of acute myocardial infarction have demonstrated a reduction in subsequent coronary events by an increased consumption of fatty fish[5] or α-linoleic acid (a precursor of n-3 fatty acids derived from vegetable sources).[6] An increase in dietary n-3 fatty acids in relation to arachidonic acid alters platelet prostaglandin and leukocyte leukotriene pathways, resulting in potentially beneficial limitation of the smooth muscle cell proliferative response underlying angioplasty restenosis.[4,7] The prevention by fish oils of proliferative atherosclerosis in the coronary-balloon-injury cholesterol-fed pig model suggested that such a benefit might accrue in humans.[8] Five trials of fish oils for the reduction of PTCA restenosis reported by 1989 suggested an overall benefit,[9-13] but deficiencies in their design and conduct prompted us to undertake a large clinical trial with sufficient power to evaluate definitively the efficacy of fish oils among patients undergoing elective PTCA.

The effort and expense of conducting the trial prompted us to maximize the utility of our undertaking by evaluating a second potentially beneficial therapy using a factorial design. This approach allows assessment of a second therapy at relatively modest additional cost and effort and represents an efficient use of resources.[14]

Various heparin fractions are effective in preventing smooth muscle cell hyperplasia after experimental arterial injury.[15-17] The antiproliferative effects appear to be in-

Received November 28, 1995; revision received April 11, 1996; accepted April 15, 1996.

From the Departments of Medicine (J.A.C., J.G., J.H., D.H.) and Clinical Epidemiology and Biostatistics (J.A.C., R.R., M.G., J.H.), McMaster University, and the Departments of Medicine, University of Ottawa (B.M., J.F.M.), University of Western Ontario (K.F.), and University of Toronto (S.N., E.C.), Canada.

Correspondence to Dr John A. Cairns, Department of Medicine, Room 3W10, McMaster University Medical Centre, 1200 Main St W, Hamilton, Ontario, Canada L8N 3Z5. E-mail Cairns@FHS.CSU.McMaster.CA.

Selected Abbreviations and Acronyms

aPTT = activated partial thromboplastin time
DHA = docosahexaenoic acid
EMPAR = Enoxaparin MaxEPA Prevention of Angioplasty Restenosis
EPA = eicosapentaenoic acid
LMWH = low-molecular-weight heparin
maxEPA = fish oil capsules containing 180 mg EPA and 120 mg DHA in the form of a triglyceride (trademarked)
PTCA = percutaneous transluminal coronary angioplasty
QCA = quantitative coronary angiography

dependent of molecular size and anticoagulant effect[17]; because LMWH fractions are about one third the size of standard heparin, they may be capable of greater inhibitory activity than unfractionated heparin for an equivalent anticoagulant effect. In venous thrombosis, LMWH produces less bleeding for an equivalent antithrombotic effect than unfractionated heparin.[18] We decided to evaluate both LMWH and fish oils for the limitation of PTCA restenosis using a 2×2 factorial design.

Methods

The trial was conducted in four teaching hospitals in southern Ontario, Canada, that together perform PTCA on about 2400 patients yearly. A nurse practitioner employed in each hospital attempted to screen every patient scheduled to undergo PTCA with respect to the following inclusion criteria: a diagnostic coronary angiogram showing at least one localized coronary artery stenosis of ≥50% reduction of lumen diameter by visual analysis and age ≥18 years. The exclusion criteria were as follows: (1) certain characteristics of the coronary artery disease (culprit lesion in a saphenous bypass graft, at the site of a previously dilated restenosis, or involving the left main coronary artery; myocardial infarction <28 days previously, the presence of very unstable angina necessitating PTCA in <48 hours, or the presence of variant angina; or the use of Sones' approach); (2) excessive bleeding risk (recent peptic ulcer or gastrointestinal bleeding, platelets <100 000/mm^3, predisposition to intracranial hemorrhage, or blood pressure >180/105 mm Hg); (3) concerns specific to the use of fish oils or LMWH (fish product or LMWH allergy or hypersensitivity, a requirement for anticoagulant therapy, the use of insulin, or significant hepatic or renal disease); or (4) practical patient problems (disease therapy that might interfere with LMWH action or evaluation; concomitant disease likely to limit life span to <6 months; drug or alcohol abuse; or insurmountable geographic, social, or language barrier).

After giving informed consent, patients were randomly allocated fish oils (maxEPA capsules, supplied by R.P. Scherer, each containing 180 mg EPA and 120 mg DHA in the form of a triglyceride), six capsules given three times daily with meals (total, 5.4 g/d n-3 polyunsaturated fatty acids), or identical-appearing capsules of corn oil placebo. The intent was to administer the capsules for not <7 days before PTCA and to continue them for 4 months. Patients with unstable angina were eligible for study entry, provided that the investigator believed that fish oils/placebo could be administered for at least 48 hours before PTCA.

All patients received full-dose standard heparin intravenously during PTCA and were maintained with an aPTT at 1.5 to 2 times control until about 4 hours before removal of the groin sheath. Those patients judged to have had successful PTCAs (residual stenosis <50% diameter by visual assessment in at least one vessel in which PTCA was attempted and no peri-procedural infarct, death, repeated PTCA, or coronary artery bypass graft) underwent a second randomization to LMWH (enoxaparine, supplied by Rhône Poulenc Rorer in prepackaged syringes) self-administered subcutaneously 30 mg BID for 6 weeks or standard therapy. No placebo injections were given to the control group. This group comprised the study cohort of interest and were the only patients followed subsequently.

Patients were reevaluated at weeks 2, 6, and 12, and follow-up coronary angiography was scheduled at 18±2 weeks (mean±SD), at which point participation in the trial would be complete. Physicians were asked to avoid repeated coronary angiography before the 16-to-20-week window if possible and consistent with the patient's best interests. If follow-up angiography was clinically required before 16 weeks and visual examination revealed any study lesion stenosed to <50%, the study drug was continued, and every effort was made to obtain a follow-up angiogram in the 16-to-20-week window. If no subsequent angiogram was done, the results obtained at the early angiogram were to be used in the determination of restenosis. If every study lesion was stenosed ≥50% at angiography before 16 weeks, the study treatment was stopped.

Coronary Angiography, PTCA, and Quantitative Stenosis Severity

Twenty-four hours before PTCA, patients began aspirin 325 mg/d (continued throughout follow-up) and a calcium antagonist (continued at the discretion of the attending cardiologist). Nitroglycerin (100 μg IC) was administered before angiography with the largest-acceptable caliber diagnostic catheter. At least two orthogonal views of each lesion to be dilated were filmed with a 7-in image intensifier and careful centering. Exact angles and distances were recorded for each lesion dilated. Standard heparin (10 000 IU IA) was given just before balloon insertion, repeated (5000 IU) every half hour to hour, and maintained (aPTT, 1.5 to 2) until about 4 hours before sheath removal. After dilatation, repeat cinefilms were recorded to duplicate the pre-PTCA projections, caliber of diagnostic catheter, and intracoronary nitroglycerin. Identical approaches were followed for the 18-week follow-up angiogram.

Quantitative measurements were made at the Ottawa Heart Institute by use of a customized Siemens Digiltron 3.61 analytical program. An Arripro projector was used to optimally magnify the image 2.8 times and to allow centering of the region of interest. From the projections indicated by the angiographer, end-diastolic frames were chosen from the cardiac cycles with best opacification of a given segment, converted to video, and digitized. Operator and computer interaction was used to draw a centerline through the area of interest, allowing edge detection and measurement of the minimal lumen diameter and a reference diameter within 1 cm proximal or distal to the lesion. Calibration markers embedded in a transparent plate placed over the image intensifier tube allowed determination of a calibration line and calculation of vessel diameters in millimeters. The diameters of each segment were calculated from the mean of two orthogonal views. Film sets (before, after, and follow-up) were analyzed at the same time by a single technologist blinded to the treatment allocation. The validity of the system had been assessed previously by filming of dye-filled Plexiglas channels, yielding a test-retest SD of 0.054 mm and a correlation of .997 with the true diameter. All measurements in the study were done by a single technologist. Replicate measures on the same patient film yielded coefficients of variation of the minimal lumen diameter of .19, .15, and .17, whereas reliabilities, expressed as interclass correlation, were 85% to 95%.

We planned to conduct both patient-based and lesion-based analyses of restenosis. In the patient-based analysis, patients were classified as having restenosis if any of their successfully dilated lesions met the definition for restenosis. The lesion-based analyses treated each successfully dilated lesion as a separate observation. The primary definition of restenosis for a lesion was a loss of ≥50% of the gain of luminal diameter achieved by PTCA based on the mean stenotic diameters of the available orthogonal views. In some situations, the post-PTCA image did not allow the quantitative assessment of gain or occasionally failed to indicate any quantitative gain. In these situations, a secondary def-

inition of restenosis was used that required percentage stenosis (with respect to a reference diameter within 1 cm of the lesion) to be ≥50% at follow-up.

Statistical Analysis

We postulated a restenosis rate of 30% in the untreated patients and sought to detect a relative reduction of 50% in this rate. Choosing a two-sided α of 0.05 and β of 0.10, we calculated a sample size of 148 patients per group (total, 592) available for follow-up angiography, necessitating the randomization of >800 patients. Differences between the treatment groups for the various clinical and laboratory outcome variables were tested by the χ^2 test or t statistic. A patient was considered to have restenosis if any successfully dilated lesion was restenosed at follow-up. The restenosis rates in the patient-based analysis were compared by use of the Mantel-Haenszel test stratified by the number of successfully dilated vessels. A lesion-based analysis included only lesions for which QCA was available and compared rates of restenosis by use of a Mantel-Haenszel χ^2 test. Mean minimal lumen diameters (also lesion-based) were compared by use of ANOVA.

Results

Patient Recruitment

The patients were recruited between June 1990 and June 1993, and the last follow-up coronary angiogram was done in November 1993. There were 814 patients randomized to fish oils/placebo, which they received for a minimum of 2 days and a median of 6 days before PTCA (Table 1). The subgroup of patients with unstable angina received the study treatment for a minimum of 2 days and a median of 3 days before PTCA. Of patients randomized to fish oils/placebo, 7% were subsequently found to be ineligible or had their PTCAs canceled for a variety of reasons. PTCA was attempted in 756 patients and was successful in 668 (88%), among whom there were 3 peri-procedural events and 12 patients who withdrew for other (mainly consent) reasons. There were 653 patients who continued to receive fish oils/placebo and then were randomized to LMWH or control. Intravenous heparin was discontinued on the day of PTCA in 5% of patients, on the morning after PTCA in 83%, and later than the morning after PTCA in 12%. The mean interval between stopping intravenous heparin and beginning LMWH was 10 hours.

Study Population

The four groups resulting from the second randomization were quite comparable, with no statistically significant differences in the proportions of any relevant baseline characteristics (Table 2). There were no statistically significant differences among the proportions of various characteristics and distributions of the successfully dilated lesions among the four treatment groups (Table 3).

Compliance

Fish oils/placebo capsules were permanently discontinued in 100 patients (15%) before the normal end of study at 18 weeks (median duration, 9 weeks). The rate of early discontinuation was similar in active and placebo fish oils groups. LMWH injections were discontinued in 68 of 325 patients (21%) before the normal end of treatment at 6 weeks (median duration, 1 week). Most early discontinuations were attributed to intercurrent ischemic events, adverse experiences, or the patient's lack of cooperation. Before cessation of study therapy (either early or at normal end of treatment), 77% of patients consumed ≥90% of their study capsules, and 88% of patients used ≥90% of their LMWH syringes.

TABLE 1. Patient Recruitment and Follow-up

	n	% of Preceding Denominator
Meet eligibility criteria	1242	
Randomized to fish oils/placebo	814	65
PTCA attempted	756	93
PTCA successful	668	88
Randomized to LMWH/control	653	98
Evaluable follow-up QCA	625	96

Clinical Events and Complications

There were no significant differences in ischemic events among the treatment groups. Bleeding was less frequent in patients taking fish oils than those taking placebo and was more frequent in patients taking LMWH than among control subjects. Most bleeding was mild, leading to permanent discontinuation of study medication in only six patients (0.9%), and no transfusions were required. Most spontaneous bleeding was characterized by bruising, and most peri-procedural bleeding consisted of excessive oozing at the femoral puncture site. Gastrointestinal side effects, most commonly bloating and burping, were reported more often by patients taking fish oils than those taking placebo (37% versus 30.8%, $P=.07$) but only occasionally resulted in early withdrawal from study medication. Complaints related to the LMWH injection site were reported by 24.6% of patients, sometimes leading to early withdrawal from study medication. Table 4 summarizes the clinical and laboratory outcomes during follow-up.

A comparison of laboratory test mean changes from baseline to 18 weeks between the fish oils and placebo groups yielded statistically significant differences only in hepatic enzymes and serum triglycerides. Both aspartate aminotransferase and alanine aminotransferase were increased by 11% to 12% at 18 weeks with fish oils, whereas the levels in patients in the placebo group decreased slightly. As expected, fish oils reduced serum triglycerides by about 33%; by comparison, the triglycerides of placebo patients decreased by 9% over the 18-week period. LMWH did not significantly affect any of the laboratory tests.

Angiographic Outcomes

Follow-up angiograms yielding QCA measurements were available for 625 of the 653 patients (96%): 49 (8%) were done before 16 weeks for clinical reasons, 461 (74%) fell within the protocol window of 16 to 20 weeks, and 115 (18%) were done late. Of the 28 patients for whom QCA measurements were not available, 6 had technically uninterpretable angiograms, 20 refused a follow-up angiogram, and 2 patients died. The 653 patients had a total of 894 successfully dilated lesions; follow-up QCA measurements were available for 824 (92%).

In Table 5, the primary efficacy comparison for fish oils is based on the row totals; for LMWH, it is based on the column totals. From the patient-based analysis, 46.5% of patients treated with fish oils experienced restenosis of one or more successfully dilated lesions compared with 44.7% of placebo patients (1.7% absolute difference; 95% CI±7.8%, $P=.6$). The restenosis rates with and without

TABLE 2. Baseline Data of Patients Undergoing Second Randomization

	Fish Oil (n=326)	Placebo (n=328)	LMWH (n=325)	Control (n=328)	All (n=653)
Male, %	83	82	83	82	82
Mean age, y	57	56	57	56	57
Range, y	31-78	29-76	34-78	29-77	29-78
Current angina, %	98	98	99	97	98
Current CHF, %	4	2	3	3	3
Past MI, %	48	51	48	52	50
Past CABG, %	7	5	6	7	6
Past PTCA, %	11	12	14	9	12
Past stroke/TIA, %	2	1	1	1	1
Past GI bleeding, %	1	3	3	1	2
Past peptic ulcer, %	7	3	6	4	5
Coronary risk factors					
Hypertension, %	31	37	33	35	34
DM, %	10	9	10	9	9
Increase in cholesterol, %	49	49	46	51	49
Family history, %	44	49	47	47	47

CHF indicates congestive heart failure; MI, myocardial infarction; TIA, transient ischemic attack; GI, gastrointestinal; and DM, diabetes mellitus.

LMWH were almost identical ($P=.8$). The formal test of interaction (ie, the tendency for the effect of one treatment to depend on the presence or absence of the other) was not significant.

The results for the lesion-based analysis (Table 5) show much the same picture. The slight variation in restenosis rates over the four treatment combinations is quite consistent with the null hypothesis of "no real difference" as manifest by nonsignificant P values for all comparisons.

The Figure displays the cumulative distributions of mean minimal lumen diameters for successfully dilated lesions before PTCA, after PTCA, and at follow-up. The corresponding tabulated means and SDs are summarized in Table 6, which also shows the equivalent summary statistics for the reference diameters. The virtual coincidence of the cumulative distributions and the similarity of tabulated means at each time point show no evidence of a treatment effect for either fish oils or LMWH.

Discussion

In this randomized, factorial trial of fish oils versus placebo and of LMWH versus control among patients undergoing PTCA, there was no reduction in restenosis rate with either agent alone or in combination. The outcome was particularly unexpected for fish oils, given a strong experimental rationale and a meta-analysis of previous trials suggesting a reduction of the rate of restenosis.[4]

The trials reported before[9-13] and during[19-22] the conduct of EMPAR had defects in design, including relatively low dose of n-3 fatty acids,[21,22] short or absent preprocedural treatment period,[10,12,19,22] follow-up assessment that used nonquantitative angiography[9-11,19] or nonangiographic criteria,[12,13,19] and inadequate sample size.[9-13,19-22] The EMPAR Trial was designed to avoid these defects.

The absence of benefit in this study probably is not attributable to the dose of fish oils or the duration of treatment before PTCA. The dose of fish oils used in the EMPAR Study is sufficient to profoundly change the ratio of n-3 to n-6 fatty acids in the platelet membrane, and the 7-day pre-PTCA treatment period is sufficient for the majority of the eventual n-3 substitution and alteration of platelet function to occur.[23] Changes in red blood cell membranes may occur more slowly.[24] The 653 patients with visually successful PTCA, of whom 92% had an evaluable follow-up QCA, provided statistical power sufficient that if fish oils could indeed reduce angioplasty restenosis, the likelihood of failing to detect a clinically important reduction of restenosis is extremely low.

Since the completion of EMPAR, an additional study of fish oils for the prevention of angioplasty restenosis has been published, also with negative results.[24] The study randomized 551 patients to 6.9 g/d n-3 fatty acids for 12 to 14 days before and 6 months after PTCA. QCA in 447 evaluable patients revealed a restenosis rate of 46% for placebo and 52% for fish oils, even with a higher dose of fish oils for a longer period of time than was used in the present study. It therefore seems clear that the biochemical changes resulting from fish oil supplementation do not lead to a reduction in angioplasty restenosis.

The fish oils option can now be confidently set aside. Despite an appealing experimental rationale, evidence of possible benefit in chronic coronary artery disease, and early promise in small clinical trials in patients undergoing angioplasty, this therapy has not been demonstrated to offer a benefit in the two most recent and optimally designed clinical trials involving a total of 1073 patients, more than were studied in all previous trials. A formal meta-analysis based on the summary results from the 10 published randomized controlled trials among 2106 patients fails to indicate any reduction of restenosis by fish oils (odds reduction, 1%; $P=.95$). It appears that the epidemiological observations of the reduced rates of ischemic heart disease outcomes and the post–acute myocardial infarction clinical trial results, while possibly being relevant to the progression and development of complications in patients with coronary atherosclerosis, are not relevant to the rapid smooth muscle cell proliferation pathogenesis of PTCA restenosis.

Apart from one small trial of fragmin that reported a favorable trend,[25] trials of heparin to prevent PTCA restenosis have been negative,[26-30] including the only other reported trial of sustained LMWH after angioplasty.[29] In that trial, patients were randomized to enoxaparine 40 mg SC

TABLE 3. Distribution and Nature of the Successfully Dilated Lesions

	Fish Oils (n=325)	Placebo (n=328)	LMWH (n=325)	Control (n=328)	All (n=653)
Distribution of number of lesions, %					
1	70	69	66	73	70
2	25	24	27	22	24
≥3	5	7	7	5	6
Distribution of number of vessels, %					
1	88	89	89	89	89
2	11	11	11	11	11
3	0.3	0.00	0.3	0.00	0.00
Number of lesions	439	455	460	454	894
Distribution of vessels, %					
LAD	42	43	44	41	42
RCA	37	36	37	36	37
Circumflex	21	21	19	23	21
Nature of lesion, %					
100% Occlusion	5	4	4	5	5
Previous restenosis	9	6	7	8	7
Neither	86	90	89	87	88

LAD indicates left anterior descending coronary artery; RCA, right coronary artery.

daily or placebo for 28 days. QCA in 394 patients at 24±4 weeks after PTCA revealed restenosis rates of 52% with enoxaparine and 51% with placebo. The EMPAR Study was larger; the dose of enoxaparine was 50% greater and given twice daily; the heparin-free interval was shorter; and the treatment was sustained 50% longer but was also negative.

A number of mechanisms could be responsible for the inhibition of experimental smooth muscle hyperplasia by heparin and its fractions. These sulfated polysaccharides may compete for endoglycosidases released by activated platelets, thereby protecting heparan sulfate on the surface of smooth muscle cells, in turn inhibiting smooth muscle mitogenesis.[31] Heparin and its derivatives also have the potential to inhibit smooth muscle mitogenesis by binding to growth factors released from cells at sites of vascular injury[32] or by a direct effect on the cell nucleus.[33]

Although in EMPAR high-dose unfractionated heparin was administered beginning ≈30 minutes before PTCA, experimental evidence exists that the antiproliferative effects of heparin are increased by pretreatment.[34] Heparin was discontinued for about 4 hours before sheath removal, creating a gap in the inhibitory effect on smooth muscle cell proliferative factors, possibly at a critical stage. On the other hand, some studies have shown that the inhibitory effects of heparin can be

TABLE 4. Clinical and Laboratory Outcomes During Follow-up

	Fish Oils (n=325), %		Placebo (n=328), %		LMWH (n=325), %		Control (n=328), %		All (n=653), %
Ischemic events									
Unstable angina	10.5		8.2		9.8		8.8		8.5
Myocardial infarction	0.9		1.5		1.8		0.6		1.2
Stroke	0.00		0.00		0.00		0.00		0.00
Death	0.9		0.3		0.9		0.3		0.6
CABG	2.8		0.9		1.5		2.1		1.8
PTCA	20.3		19.5		19.7		20.1		19.9
Adverse events									
Bleeding									
Any	5.2*		11.6		11.1*		5.8		8.4
Permanent discontinuation of study medications	0.9		0.9		1.8		0.00		0.9
GI symptoms	37.5*		30.8		33.9		34.5		34.2
	Baseline	18 wk	Baseline	18 wk	Baseline	18 wk	Baseline	18 wk	
Selected laboratory values									
AST, U/L	23.5	26.3*	22.8	21.8	22.8	24.0	23.6	24.1	
ALT, U/L	26.5	29.7*	25.5	22.5	25.7	26.1	26.3	26.3	
TG, mmol/L	2.56	1.72*	2.52	2.30	1.47	1.94	2.71	2.09	
TC, mmol/L	5.91	5.59	5.98	5.75	5.91	5.59	5.97	5.75	
HDL cholesterol, mmol/L	1.04	1.04	1.05	1.05	1.05	1.05	1.04	1.04	
LDL cholesterol, mmol/L	3.83	3.71	3.87	3.67	3.83	3.84	3.87	3.74	

CABG indicates coronary artery bypass graft; GI, gastrointestinal; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TG, triglycerides; and TC, total cholesterol.
*$P<.05$ for comparison of fish oils to placebo or LMWH to placebo.

TABLE 5. Comparison of Restenosis Rates at Follow-up

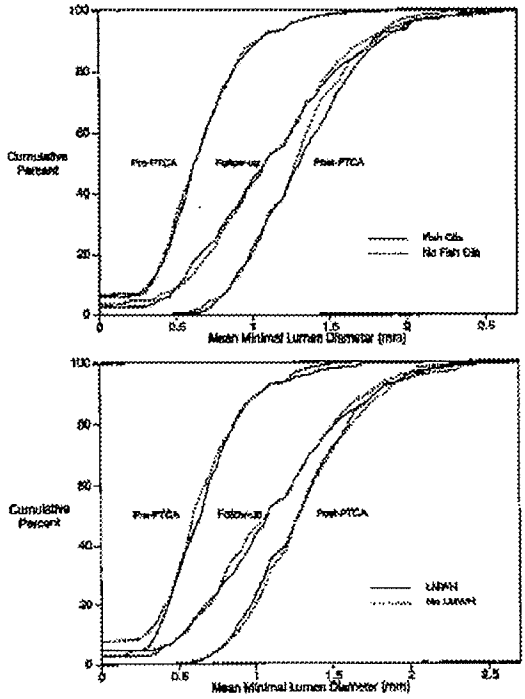
	LMWH, n (%)		
Fish Oil	No	Yes	Combined, n (%)
Patient-Based Analysis			
No	71/155 (45.8)	69/158 (43.7)	140/313 (44.7)
Yes	72/160 (45.0)	73/152 (48.0)	145/312 (46.5)
Combined	143/315 (45.4)	142/310 (45.8)	285/625 (45.6)
Lesion-Based Analysis			
No	84/202 (41.6)	77/214 (36.0)	161/416 (38.7)
Yes	82/209 (39.2)	80/199 (40.2)	162/408 (39.7)
Combined	166/411 (40.4)	157/413 (38.0)	323/824 (39.2)

For patient-based analysis, P (fish oil)=.60; P (LMWH)=.80; and P (interaction)=.36. For lesion-based analysis, P=.78, P=.49, and P=.33 for fish oil, LMWH, and interaction, respectively.

achieved even when it is started after vessel injury.[34] Animal experiments indicate that the antiproliferative effects of heparin are dose dependent.[17] Recent studies of LMWH in human arterial diseases have demonstrated that doses much higher than those used in trials of venous thrombosis were safe[35] and would therefore be feasible after PTCA. It is also conceivable that the treatment period of 6 weeks in this study was insufficient to realize the benefits of LMWH.

With the use of a factorial design, the patients were randomized to fish oils or identical-appearing placebo and subsequently randomized to LMWH or standard therapy with no LMWH placebo. Because the comparison of restenosis rates was based on the QCA results performed by a technologist blinded to treatment allocations, minimal bias was anticipated; accordingly, the patient discomfort that would have arisen from placebo injections was judged to be unwarranted.

The potential benefit from LMWH is unclear. The two negative studies involving 1019 patients leave little doubt as to the lack of efficacy with a dose of up to 60 mg/d begun at the time of angioplasty and continued for 6 weeks. Although the impressive results in animal experiments may prompt further clinical trials with appropriate modifications in design, in a recent study in baboons, high-dose LMWH failed to inhibit intimal hyperplasia in a balloon angioplasty model, suggesting that species differences may preclude a beneficial effect of LMWH in humans.[36]



Cumulative frequency distribution curves of the mean minimal lumen diameters of lesions undergoing visually successful PTCA measured before PTCA, immediately after PTCA, and at follow-up. Top, fish oils and placebo groups; bottom, LMWH and control groups.

Is the goal of reduction of restenosis attainable with any intervention? Although numerous trials have failed to demonstrate benefit from steroids, ACE inhibitors, antiplatelet agents, cholesterol-lowering agents, or oral anticoagulants,[3] recent studies suggest that stent implantation may be effective in selected patients,[37,38] and most recently the platelet-derived growth factor antagonist triazolopyrimidine[39] appears to be effective. A meta-analysis of trials

TABLE 6. QCA Measurements of Minimal Lumen Diameters and Associated Reference Diameters for All Lesions

	Fish Oils (n=408)		Placebo (n=416)	
Study Period	Reference Diameter, mm	Minimal Lumen Diameter, mm	Reference Diameter, mm	Minimal Lumen Diameter, mm
Before PTCA	2.53±0.59	0.67±0.31	2.50±0.54	0.67±0.31
Immediately after PTCA	2.50±0.56	1.33±0.39	2.46±0.54	1.30±0.39
Follow-up PTCA	2.45±0.57	1.12±0.50	2.39±0.47	1.10±0.47
Initial gain	−0.03±0.28	0.66±0.42	−0.04±0.29	0.63±0.40
Late loss	0.06±0.29	0.21±0.43	0.07±0.40	0.20±0.47
	Enoxaparine (n=413)		Control (n=411)	
Before PTCA	2.49±0.56	0.69±0.31	2.54±0.57	0.65±0.31
Immediately after PTCA	2.46±0.56	1.30±0.38	2.49±0.56	1.33±0.41
Follow-up PTCA	2.39±0.62	1.12±0.50	2.44±0.57	1.10±0.47
Initial gain	−0.03±0.25	0.62±0.40	−0.05±0.26	0.68±0.42
Late loss	0.07±0.38	0.18±0.43	0.06±0.33	0.23±0.48

Values are mean±SD.

of calcium antagonists suggests a benefit,[40] although a well-designed single trial is required to provide data that will convince practicing cardiologists. Restenosis continues to complicate 40% to 50% of PTCA procedures; ongoing attacks on this problem are warranted and should be undertaken.

Appendix

The following hospitals and investigators participated in the EMPAR Study. The number of patients enrolled at each center is given in parentheses: Ottawa (Canada) Heart Institute (273): Brian Morton, Jean François Marquis, Bill Williams, Donald Beanlands, Bernie Larocque, Laurie Jozwiak, and Joanne Taylor; Victoria General Hospital, London, Canada (190): Keith Fimnie, Ian Penn, Robert Brown, Brendan Foley, Joanne White, and Karen Gier; Hamilton (Canada) General Hospital (182): Douglas Holder, John Gill, Corinne O'Dell, and Chris Basset; Sunnybrook Health Sciences Centre, Toronto, Canada (169): Sal Naqvi, Eric Cohen, Gregory Mishkel, Lynn Balcsa, and Neville Arthurs.

Steering Committee: John Cairns (chairman), John Gill, Robin Roberts, Michael Gent, Jack Hirsh, Brian Morton, Keith Fimnie, Sal Naqvi, Jean François Marquis, Douglas Holder, Bernie Laroque, Eric Cohen, Robert Brown, and Ian Penn.

External Safety and Efficacy Monitoring Committee: Wayne Taylor (chairman), Graham Turpie, and Leonard Schwartz.

Coordinating and Methods Centre: Robin Roberts, Michael Gent (director), Lorrie Costantini, and Wendy Yacura.

Quantitative Coronary Angiography Committee: Brian Morton (chairman), John Gill, Douglas Holder, Jean François Marquis, and Bernie Larocque.

Acknowledgments

This work was supported by a grant-in-aid from the Heart and Stroke Foundation of Ontario and by a grant from Rhône Poulenc Rorer. We wish to express our sincere appreciation to our colleagues who performed the cardiac catheterizations and PTCAs, the nurses and technicians in the cardiac catheterization laboratories and radiology departments of the participating hospitals, and Susan Crook for secretarial support.

References

1. Ryan TJ, Bauman WB, Kennedy JW, Kereiakes DJ, King SB, McAllister BD, Smith SC, Ullyot DJ. ACC/AHA Task Force report: guidelines for percutaneous transluminal coronary angioplasty: a report of the American College of Cardiology/American Heart Association Task Force on Assessment of Diagnostic and Therapeutic cardiovascular Procedures (Committee on Percutaneous Transluminal Coronary Angioplasty). *J Am Coll Cardiol*. 1993;22:2033-2054.
2. Holmes DR, Schwartz RS, Webster MW. Coronary restenosis: what have we learned from angiography? *J Am Coll Cardiol*. 1991;17:14B-22B.
3. Hillegass WB, Ohman EM, Califf RM. Restenosis: the clinical issues. In: Topol EJ. *Textbook of Interventional Cardiology*. Philadelphia, Pa: WB Saunders Co; 1994:415-435.
4. Goodnight SH, Cairns JA, Fisher M, Fitzgerald GA. Assessment of the therapeutic use of n-3 fatty acids in vascular disease and thrombosis. *Chest*. 1992;102:374S-384S.
5. Burr ML, Gilbert JF, Holliday RM, Elwood PC, Fehily AM, Rogers S, Sweetnam PM, Deadman NM. Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: Diet and Reinfarction Trial (DART). *Lancet*. 1989;2:757-761.
6. De Lorgeril M, Renaud S, Mamelle N, Salen P, Martin J-L, Monjaud I, Guidollet J, Touboul P, Delaye J. Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. *Lancet*. 1994;343:1454-1459.
7. Leaf A, Weber P. Cardiovascular effects of n-3 fatty acids. *N Engl J Med*. 1988;318:549-557.
8. Weber BH, Ockene IS, Levine PH, Cucnoud HF, Fisher M, Johnson BF, Daoud AS, Jarmolych J, Hosmer D, Johnson MH, Natale A, Vandreuil C, Hoogasian JJ. Inhibition of atherosclerosis by cod liver oil in a hyperlipidemic swine model. *N Engl J Med*. 1986;315:841-846.
9. Dehmer GJ, Popma JJ, van der Berg EK, Eichhorn EJ, Prewitt JB, Campbell WB, Jennings L, Willerson JT, Schmitz JM. Reduction in the rate of early restenosis after coronary angioplasty by a diet supplemented with n-3 fatty acids. *N Engl J Med*. 1988;319:733-740.
10. Grigg LE, Kay TWH, Valentine PA, Larkins R, Flower DJ, Manolas EG, O'Dea K, Sinclair AJ, Hopper JL, Hunt D. Determinants of restenosis and lack of effect of dietary supplementation with eicosapentaenoic acid on the incidence of coronary artery restenosis after angioplasty. *J Am Coll Cardiol*. 1989;13:665-672.
11. Reis GJ, Boucher TM, Sipperly ME, Silverman DI, McCabe CH, Baim DS, Sacks FM, Grossman W, Pasternak RC. Randomised trial of fish oil for prevention of restenosis after coronary angioplasty. *Lancet*. 1989;2:177-181.
12. Milner MR, Gallino RA, Leffingwell A, Pichard AD, Brooks-Robinson S, Rosenberg J, Little T, Lindsay J. Usefulness of fish oil supplements in preventing clinical evidence of restenosis after percutaneous transluminal coronary angioplasty. *Am J Cardiol*. 1989;62:294-299.
13. Slack JD, Pinkerton CA, Van Tassel J, Orr CM, Scott M, Allen B, Vasser WK. Can oral fish oil supplement minimize restenosis after percutaneous transluminal angioplasty? *J Am Coll Cardiol*. 1987;9:64A. Abstract.
14. Peto R. Clinical trial methodology. *Biomedicine*. 1978;28:S49-S57.
15. Clowes AW, Karnowsky MJ. Suppression by heparin of smooth muscle cell proliferation in injured arteries. *Nature*. 1977;265:625-626.
16. Guyton RJ, Rosenberg RD, Clowes AW, Karnovsky MJ. Inhibition of rat arterial smooth muscle cell proliferation by heparin: in vivo studies with anticoagulant and nonanticoagulant heparin. *Circ Res*. 1980;46:625-634.
17. Currier JW, Pow TK, Handenschild CC, Minihan AC, Faxon DP. Low molecular weight heparin (enoxaparin) reduces restenosis after iliac angioplasty in the hypercholesterolemic rabbit. *J Am Coll Cardiol*. 1991;17(suppl B):118B-125B.
18. Hirsh J, Levine MN. Low molecular weight heparin. *Blood*. 1992;79:1-17.
19. Nye ER, Ilsley CDJ, Ablett MB, Sutherland WHF, Robertson MC. Effect of eicosapentaenoic acid on restenosis rate, clinical course and blood lipids in patients after percutaneous transluminal coronary angioplasty. *Aust N Z J Med*. 1990;20:549-552.
20. Bairati I, Roy L, Meyer F. Double-blind, randomized, controlled trial of fish oil supplements in prevention of recurrence of stenosis after coronary angioplasty. *Circulation*. 1992;85:950-956.
21. Kant U, Sanghvi S, Babl VK, Dev V, Wasir HS. Fish oil supplements for prevention of restenosis after coronary angioplasty. *Int J Cardiol*. 1992;34:87-93.
22. Bellamy CM, Schofield PM, Faragher EB, Ramsdale DR. Can supplementation of diet with omega-3 polyunsaturated fatty acids reduce coronary angioplasty restenosis rate? *Eur Heart J*. 1992;13:1626-1631.
23. Knapp HR, Reilly IAG, Alessandrini P, FitzGerald GA. In vivo indexes of platelet and vascular function during fish oil administration in patients with atherosclerosis. *N Engl J Med*. 1986;314:937-942.
24. Leaf A, Jorgensen MB, Jacobs AK, Cote G, Schoenfeld DA, Jeheer J, Weiner BH, Slack JD, Kellet MA, Reimer AE, Weber PC, Mahrer PR, Rosseouw JE. Do fish oils prevent restenosis after coronary angioplasty? *Circulation*. 1994;90:2248-2257.
25. Schmidt T, Tebbe U, Schrader J, Brune S, Kreuzer H. Pharmacological therapy after coronary angioplasty: early experience with low molecular weight heparin for prophylaxis of reocclusion. *Klin Wochenschr*. 1990;68:294.
26. Ellis SG, Roubin GS, Wilentz J, Douglas JS Jr, King SB III. Effect of 18 to 24 hour heparin administration for prevention of restenosis after uncomplicated coronary angioplasty. *Am Heart J*. 1989;117:777-782.
27. De Vries CJ, Michels HR, Emmanuelsson M, El Gamel MIH. Does administration of low molecular weight heparin after angioplasty affect restenosis? *Eur Heart J*. 1991;12(suppl):386.
28. Lehrman KG, Donia RJ, Feuer AJM, Hall PX, Hoang DT. Paradoxical increase in restenosis rate with chronic heparin use: final results of a randomized trial. *J Am Coll Cardiol*. 1991;17:181A. Abstract.
29. Faxon DP, Spiro TE, Minor S, Coté G, Douglas J, Gottlieb R, Califf R, Dorosti K, Topol E, Gordon JB, Ohman M, and the ERA Investigators. Low molecular weight heparin in prevention of restenosis after angioplasty: results of Enoxaparine Restenosis (ERA) Trial. *Circulation*. 1994;90:908-914.
30. Brack MJ, Ray S, Chauhan A, Fox J, Hubner PJB, Schofield P, Hadley A, Gershlick AH, for the SHARP Investigators. The Subcutaneous Heparin and Angioplasty Restenosis Prevention (SHARP) Trial: results of a multicenter randomized trial investigating the effects of high dose un-

fractionated heparin in angiographic restenosis and clinical outcome. *J Am Coll Cardiol*. 1995;26:947-954.
31. Castellot JJ, Favreau LV, Karnovsky MJ, Rosenberg RD. Inhibition of vascular smooth muscle cell growth by endothelial cell-derived heparin: possible role of a platelet endoglycosidase. *J Biol Chem*. 1982;257:1256-1260.
32. Majesky MW, Schwartz SM, Clowes MM, Clowes AW. Heparin regulates smooth muscle S phase entry in the injured rat carotid artery. *Circ Res*. 1987;61:246-300.
33. Castellot JJ Jr, Cochran DL, Karnovsky MJ. Effect of heparin on vascular smooth muscle cells, I: cell metabolism. *J Cell Physiol*. 1985;124:21-28.
34. Reilly CF, Fritze LMS, Rosenberg RD. Heparin inhibition of smooth muscle cell proliferation: a cellular site of action. *J Cell Physiol*. 1986;129:11-19.
35. Gurfinkel EP, Manos EJ, Mejail RI, Cerda MA, Duronto EA, Garcia CN, Daroca AM, Mautner B. Low molecular weight heparin versus regular heparin or aspirin in the treatment of unstable angina and silent ischemia. *J Am Coll Cardiol*. 1995;26:313-318.
36. Geary RL, Koyama N, Wang TW, Vergel S, Clowes AW. Failure of heparin to inhibit intimal hyperplasia in injured baboon arteries: the role of heparin-sensitive and -insensitive pathways in the stimulation of smooth muscle cell migration and proliferation. *Circulation*. 1995; 91:2972-2981.
37. Serruys PW, de Jaegere P, Kiemeneij F, Macaya C, Rutsch W, Heyndrickx G, Emanuelsson H, Marco J, Legrand V, Materne P, Belardi J, Sigwart U, Colombo A, Goy JJ, van den Heuvel P, Delcan J, Morel M-A, for the Benestent Study Group. A comparison of balloon-expandable-stent implantation with balloon angioplasty in patients with coronary artery disease. *N Engl J Med*. 1994;331:489-495.
38. Fischman DL, Leon MB, Baim DS, Schatz RA, Savage MP, Penn I, Detre K, Veltri L, Ricci D, Nobuyoshi M, Cleman M, Heuser R, Almond D, Teirstein BS, Fish D, Colombo A, Brinker J, Moses J, Shaknovich A, Hirshfeld J, Bailey S, Ellis S, Rake R, Goldberg S, for the Stent Restenosis Study Investigators. A randomized comparison of coronary-stent placement and balloon angioplasty in the treatment of coronary artery disease. *N Engl J Med*. 1994;331:496-501.
39. Maresta A, Balocelli M, Cantini L, Casari A, Chioin R, Fabbri M, Fontanelli A, Preti PAM, Repetto S, DeServi S, Varani E, for the STARC Investigators. Trapidil (triazolopyrimidine), a platelet-derived growth factor antagonist, reduces restenosis after percutaneous transluminal coronary angioplasty: results of the randomized, double-blind STARC study. *Circulation*. 1994;90:2710-2715.
40. Hillegass WB, Ohman EM, Leimberger JD, Califf RM. A meta-analysis of randomized trials of calcium antagonists to reduce restenosis after coronary angioplasty. *Am J Cardiol*. 1994;73:835-839.

A1442-1443

REDACTED

ELSEVIER

Cardiovascular Research 63 (2004) 617–624

Cardiovascular Research

www.elsevier.com/locate/cardiores

Long-term effects of polymer-based, slow-release, sirolimus-eluting stents in a porcine coronary model

Andrew J. Carter[a,*], Meenakshi Aggarwal[b], Gregory A. Kopia[c], Fermin Tio[d], Philip S. Tsao[b], Ron Kolata[e], Alan C. Yeung[b], Gerald Llanos[c], John Dooley[c], Robert Falotico[c]

[a] *Providence Heart and Vascular Institute, Providence St. Vincent Medical Center, 9205 SW Barnes Road, Portland, OR 97225-5218, USA*

[b] *Stanford University Medical Center, Stanford, CA, USA*

[c] *Cordis Co., Warren, NJ, USA*

[d] *University of Texas at San Antonio Health Sciences Center, San Antonio, TX, USA*

[e] *Ethicon Endo-Surgery, Inc., Cincinatti, OH, USA*

Received 1 March 2004; received in revised form 31 March 2004; accepted 26 April 2004

Available online 5 June 2004

Time for primary review 19 days

Abstract

Background: Stent-based delivery of sirolimus (SRL) has shown reduction in neointimal hyperplasia and restenosis. The purpose of this study was to evaluate the chronic vascular response and the expression of cell cycle regulators after SRL-eluting stent implantation in a porcine coronary model. **Methods:** Forty-nine pigs underwent placement of 109 oversized stents (control, $n=54$, SRL (140 μg/cm^2), $n=55$) in the coronary arteries with histologic analysis and Western blot (PCNA, p27^{kip1}, CD45, MCP-1, IL-2, IL-6, TNF-β) at 3, 30, 90 or 180 days. **Results:** At 3 days, the mean thrombus area was similar for control (0.38 ± 0.19 mm^2) and SRL (0.29 ± 0.09 mm^2) stents. After 30 days, the mean neointimal area was significantly less for the SRL (1.40 ± 0.35 mm^2) versus the control stents (2.94 ± 1.28 mm^2, $p<0.001$). At 90 and 180 days, the mean neointimal area was similar for the SRL (3.03 ± 0.92 and 3.34 ± 0.99 mm^2) as compared with control stents (3.45 ± 1.09 and 3.65 ± 1.23 mm^2). Western blot analysis demonstrated an increased expression of p27^{kip1} in the vessel wall at 90 days for the SRL versus control stents ($p=0.05$) but with increased levels of PCNA in the SRL as compared with control stents ($p=0.003$). **Conclusion:** SRL-eluting stents favorably modulate neointimal formation for 30 days in the porcine coronary model. Long-term inhibition of neointimal hyperplasia is not sustained presumably due to delayed cellular proliferation despite increased levels of the cyclin-dependent kinase p27^{kip1} in the vessel wall.

Keywords: Stents; Restenosis; Smooth muscle; Cell cycle

This article is referred to in the Editorial by A. Lafont (pages 575–576) in this issue.

We have previously documented that stent-based delivery of sirolimus (SRL) suppressed neointimal hyperplasia at 28 days presumably via inhibition of cell proliferation and the expression of inflammatory cytokines in the porcine coronary model [1]. These encouraging data provided the impetus to embark on long-term experimental studies to determine efficacy, biocompatibility as well as to assess for potential indicators of vascular toxicity such as medial necrosis, severe inflammation, aneurysm formation, delayed endothelialization, and stent thrombosis.

The purpose of the present study was to determine the long-term effects of SRL eluting stents on neointimal formation in the porcine coronary model. The temporal effects of SRL stents on neointimal formation and other components of arterial repair were assessed by qualitative and quantitative histopathology at 3, 30, 90 and 180 days. Additional experiments were conducted to measure biological markers of cell proliferation (PCNA), cell cycle activation (p27^{kip1}), and inflammation (MCP-1, CD45, IL-2, IL-6, TNF-β) at 3, 30 and 90 days by Western

* Corresponding author. Tel.: +1-503-2165206; fax: +1-503-2162112.
E-mail address: acarter@providence.org (A.J. Carter).

0008-6363/$ - see front matter © 2004 European Society of Cardiology. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.cardiores.2004.04.029

blot analysis to determine the mechanism by which the SRL eluting stent inhibits neointimal formation.

1. Methods

1.1. Experimental studies

Stainless steel balloon expandable tubular stents (BX Velocity™, Cordis, Warren, NJ) were coated with a thin layer of a co-polymer containing ≈ 140 μg/cm^2 of SRL (Wyeth-Ayerst, Princeton, NJ) a slow (Cypher™, Cordis) release delivery system [1]. Drug elution is >90% complete after 12 weeks for this drug eluting system. Bare metal BX Velocity™ stents served as controls. All stents were individually packaged, coded with a serial number on the packaging label and ETO sterilized. The identity of each serial number was known only by the sponsor to permit deployment and analysis of each stent in a blinded fashion.

Experimental studies were conducted after IACUC approval in accordance with NIH and AHA guidelines for animal research. Forty-nine (≈ 50 to 75 kg) pigs (34 Yucatan mini-pigs, 79 stents for histopathology analysis; 15 Juvenile Yorkshire pigs, 30 stents for Western Blot analysis) underwent placement of 109 stents (control, $n=54$, SRL, $n=55$) in the left anterior descending, circumflex or right coronary artery. The guiding catheter was used as a reference in order to obtain a 1.1–1.3:1 stent to artery ratio as compared with the baseline vessel diameter. Animals were allowed to recover and returned to care facilities where they received a normal diet, aspirin 325 mg daily for the duration of the study and clopidogrel 75 mg daily for 2 months. At 3 days ($n=10$), 30 days ($n=13$), 90 days ($n=16$), or 180 days ($n=10$), the animals were euthanized after completion of coronary angiography to obtain specimens for histological analysis or Western blot of stented arterial segments.

1.2. Quantitative coronary angiography

Angiographic images of stent implants for histological analysis ($n=79$) were saved to a CD-ROM disk in a standard DICOM format. Images were analyzed on a PC-based quantitative coronary angiographic analysis software program (CCAL, Stanford University Medical Center, Stanford, CA). The guiding catheter served as a reference for calibration for all measurements. Measurements included: baseline reference vessel diameter, balloon inflated diameter, post-stent minimal lumen diameter, follow-up reference vessel diameter, follow-up minimal lumen diameter, follow-up percent diameter stenosis. The balloon to artery ratio was calculated as: the balloon inflated diameter/reference vessel diameter. The percent diameter stenosis was calculated as: $100 \times [1 - (\text{minimal lumen diameter/reference vessel diameter})]$.

1.3. Biological markers of inflammation and cell cycle activation

At 3, 30 or 90 days, SRL ($n=15$) and control ($n=15$) stent segments were removed from freshly isolated arterial specimens. The excess or loose perivascular tissue was carefully dissected from the stent. The vessel was bisected to allow extraction of the stent from the vessel wall. Tissue samples were then snap frozen in liquid nitrogen and stored at −70 °C. Vessel wall expression of PCNA (DAKO: M0879), p27^{kip1} (Santa Cruz Biotechnology: sc-528), MCP-1 (R&D Systems: MAB679, clone 23007.111), TNF-β (Boehringer Mannheim: 1141333, clone 9B9), CD45, IL-2 and IL-6 (R&D Systems: MAB114; AF652; AF686) was evaluated by Western blot analysis. Briefly, protein extracts (50 μg) were size fractionated on SDS-polyacrylamide gels, transferred to nitrocellulose membrane. Positive control for each target was run on the same gel. Membranes were incubated with an affinity purified polyclonal antibody to PCNA, p27^{kip1}, MCP-1, TNF-β, CD45, IL-2 and IL-6 respectively, washed and incubated with secondary antibody. Signals were detected by the ECL chemiluminesence detection system. Autoradiographic signals were quantified by densitometry. Tubulin was used as an internal control to ensure equal amount of protein extract in each sample. All results were compared to the aorta of the respective animal. SRL levels in the arterial wall, and the stent were determined at 90 days using HPLC [2].

1.4. Pathologic evaluation

Immediately following euthanasia, the hearts were harvested, and the coronary arteries were perfusion-fixed with 10% buffered formalin at 100 mm Hg. The stented coronary artery segments were processed for plastic embedding, staining and morphometric analysis of six sections from the proximal through the distal margin of the stent [1,3,4]. The specimens were embedded in methyl methacrylate and sections were obtained with a Beuhler isomet saw (Beuhler, Evanston, IL). The sections were then polished, mounted on a glass slide and stained with metachromatic stain. All histopathologic analysis was completed by a single independent investigator (F.T.) blinded to treatment group. Vessel morphometry (Sigmascan Morphometric Software, Jandel Scientific, San Rafael, CA) and morphologic analysis of injury, inflammation, endothelialization, fibrin, and smooth muscle content were completed using published methods [1,3,4].

Stent endothelialization score was defined as the extent of the circumference of the arterial lumen covered by endothelial cells and graded from 1 to 3 (1 = 25%, 2 = 25% to 75%, 3 = >75%). Injury score was determined by the method of Schwartz et al. [3]. Inflammation was graded as 0, none; 1, scattered inflammatory cells; 2, inflammatory cells encompassing 50% of a strut in at least 25% to 50% of the

circumference of the artery; 3, inflammatory cells surrounding a strut in at least 25% to 50% of the circumference of the artery. The intimal fibrin content was graded as 0, no residual fibrin; 1, focal regions of residual fibrin involving any portion of the artery or moderate fibrin deposition adjacent to the strut involving <25% of the circumference of the artery; 2, moderate fibrin involving >25% of the circumference of the artery or heavy deposition involving <25% of the circumference of the artery; or 3, heavy fibrin deposition involving >25% of the circumference of the artery. The intimal SMC content was scored as 1, sparse SMC density involving any portion of the artery and for moderate SMC infiltration less than the full thickness of the neointima involving <25% of the circumference of the artery; 2, moderate SMC infiltration less than the full thickness of the neointima involving >25% of the circumference of the artery or dense SMC content the full thickness of the neointima involving <25% of the circumference of the artery; or 3, dense SMC content the full thickness of the neointima involving >25% of the circumference of the artery. A positive giant cell reaction was defined as the presence of giant cells on a single section from the stent.

1.5. Statistical analysis

The morphometric measurements from each of the 4-stent sections were summed and divided by 4 to generate the mean value for each parameter within the stent. For continuous variables, such as morphometric parameters, the mean differences between treatment groups were tested with ANOVA. For morphologic parameters, scores were assigned to each of the four sections within the stented segment, the median value used as the score for the stent. The data were ranked within each cohort (3, 30, 90, or 180 days) and stratified. An ANOVA was performed on these ranks. Categorical data were compared with chi-square analysis. Data are expressed as mean ± S.D. unless otherwise stated. All statistical analysis was performed with SAS® system software.

2. Results

A total of 109 of 109 stents were successfully implanted in the coronary arteries of 49 swine. Stent migration occurred in one implant during balloon withdrawal (SRL group) that necessitated post-dilation with a 4.0-mm diameter, 20-mm-long non-compliant balloon. A total of 49 of 49 animals (100%) survived the intended study interval without clinical or angiographic stent thrombosis. The animals remained well throughout the study without abnormal temperature, weight loss, or other major health problems.

2.1. Quantitative coronary angiography

The baseline vessel diameter was similar for both SRL and control stents (range 2.55–2.94 mm). The balloon to artery ratio was similar for each group, approximately 1.2 to 1 (range 1.16–1.29 to 1). After 30 days, the SRL group had significantly less in-stent %stenosis ($-24.4 \pm 17.7\%$) versus the control stents ($-3.6 \pm 10.5\%$, $p<0.05$). At 90 and 180 days, the control (90 days, 8.7 ± 8.5; 180 days, 3.9 ± 11.4) and SRL (90 days, 2.5 ± 16.5; 180 days, 0.8 ± 9.1) stents each exhibited minimal and similar angiographic narrowing. There were no cases of greater than 50% diameter stenosis for the SRL or control stents at 3, 30, 90 or 180 days. Qualitative analysis of angiograms failed to identify intraluminal filling defects, edge effects or aneurysms for control or the SRL groups.

2.2. Histology

The histomorphometry and a semi-quantitative scoring for injury, inflammation and intimal fibrin content at 3, 30, 90 and 180 days for control and the SRL eluting stents are summarized in Tables 1–3 and Figs. 1–3. Vessel morphometry of proximal and distal adjacent non-stented sections were similar for each group at all time points (data not shown).

At 3 days, SRL and control stents had a similar appearance with fibrin-platelet deposition and acute inflammatory cells (PMNs) (Fig. 3). After 30 days, a significant (50%) reduction in neointimal area was observed for SRL stents versus control stents (Table 1, Figs. 1 and 2). The reduction in neointimal area for SRL stents resulted in 50% less cross-sectional area narrowing in comparison with control stents. The neointima for the SRL stents contained SMC, matrix proteoglycans and regions of residual fibrin deposition (Fig. 3). Infrequent regions of acellular plasma-

Table 1
Summary of histomorphometric findings at 3, 30, 90 or 180 days following placement of 79 control or SRL eluting stents in porcine coronary arteries

	Stent/IEL area	Neointimal area	% area stenosis
3 days			
Control ($n=10$)	8.31 ± 0.59	0.38 ± 0.19	4.6 ± 2.3
SRL ($n=10$)	8.61 ± 0.45	0.29 ± 0.09	3.4 ± 0.9
30 days			
Control ($n=9$)	8.48 ± 0.41	2.94 ± 1.28	34.8 ± 15.3
SRL ($n=10$)	8.23 ± 0.80	1.40 ± 0.35*,†	17.0 ± 4.3§,‡
90 days			
Control ($n=10$)	8.24 ± 1.45	3.45 ± 0.22	42.5 ± 14.9
SRL ($n=10$)	8.90 ± 1.60	3.03 ± 0.92	35.5 ± 13.5
180 days			
Control ($n=10$)	8.15 ± 0.69	3.65 ± 1.23	45.3 ± 16.5
SRL ($n=10$)	8.27 ± 0.95	3.34 ± 0.99	42.2 ± 18.1

The neointimal area reported at 3 days represents the area of thrombus measured within the stent.
*$p=0.0019$ versus 30-day control.
†$p \leq 0.0001$ versus 90- and 180-day SRL.
§$p=0.0025$ versus 30-day control.
‡$p \leq 0.015$ 90- and 180-day SRL.

Table 2
Injury in SRL and control stents by time

Group		Grade 0	Grade 1	Grade 2	Grade 3	p value
3 days	Control ($n=10$)	50% 5/10	20% 2/10	30% 3/10	0% 0/10	0.15
	SRL ($n=10$)	30% 3/10	10% 1/10	60% 6/10	0% 0/10	
30 days	Control ($n=9$)	33% 3/9	22% 2/9	45% 4/9	0% 0/10	0.49
	SRL ($n=10$)	20% 2/10	20% 2/10	60% 6/10	0% 0/10	
90 days	Control ($n=10$)	40% 4/10	10% 1/10	30% 3/10	20% 2/10	0.56
	SRL ($n=10$)	20% 2/10	10% 1/10	60% 6/10	10% 1/10	
180 days	Control ($n=10$)	30% 3/10	10% 1/10	50% 5/10	10% 1/10	0.06
	SRL ($n=10$)	10% 1/10	0% 0/10	40% 4/10	50% 5/10	

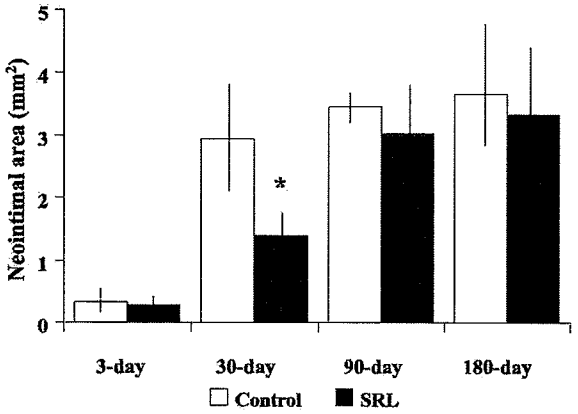


Fig. 1. Bar graphs show the temporal effects of SRL (dark) stents versus control (open) stents on thrombus and neointimal formation in porcine coronary arteries. Thrombus area is similar at 3 days for SRL and control stents. At 30 days, a significant reduction in neointimal area for SRL as compared with the control stents was observed. At 90 and 180 days, the mean neointimal area was similar for the SRL stents as compared with control stents (*$p=0.0019$ SRL versus control at 30 days). Data is mean ± S.D.

like collections were uniquely observed in the SRL stents. The SMC content was less for the SRL stents as compared with the control stents at 30 days ($p=0.01$). The media appeared intact with localized regions of compression in areas of strut-induced vessel injury. Medial necrosis was not observed in any sections from SRL or control stents. Endothelialization scores were identical (>75% complete) for SRL and control stents.

After 90 and 180 days, the mean neointimal area and %area stenosis were similar for SRL and control stents (Table 1 and Fig. 2). At 90 days, the neointima for the SRL stents contained SMC, matrix proteoglycans and regions of residual fibrin deposition (Fig. 3). Localized regions of acellular plasma-like collections, evident at 30 and 90 days, were no longer observed at 180 days in the SRL stent sections. Strut associated fibrin was more prevalent at 90 days for SRL versus control stents ($p=0.02$). At 180 days, strut-associated fibrin was not observed for SRL or control stents. SMC content score was similar for the SRL stents as compared with the control stents at 90 and 180 days. Medial necrosis was not observed in any sections from SRL or control stents. Inflammatory cells, predominantly lymphocytes, were observed in areas adjacent to stent struts for both SRL and control stents. Eosinophils were not observed in any sections from SRL or bare metal stents. At 90 days, a giant cell reaction was evident in 3 of 10 stents in the SRL and in 1 of 10 control stents ($p=0.47$). At 180 days, a giant cell reaction was evident in at least one section from 5 of 10 stents in the SRL and in 2 of 10 control stents ($p=0.23$). Endothelialization scores were identical, >75% complete, for SRL and control stents at both 90 and 180 days.

Table 3
Inflammation in SRL and control stents by time

Group		Grade 0	Grade 1	Grade 2	Grade 3	p value
3 days	Control ($n=10$)	0% 0/10	90% 9/10	10% 1/10	0% 0/10	0.33
	SRL ($n=10$)	0% 0/10	100% 10/10	0% 0/10	0% 0/10	
30 days	Control ($n=9$)	89% 8/9	11% 1/9	0% 0/10	0% 0/10	0.62
	SRL ($n=10$)	80% 8/10	20% 2/10	0% 0/10	0% 0/10	
90 days	Control ($n=10$)	90% 9/10	10% 1/10	0% 0/10	0% 0/10	0.007
	SRL ($n=10$)	30% 3/10	40% 4/10	20% 2/10	10% 1/10	
180 days	Control ($n=10$)	40% 4/10	50% 5/10	10% 1/10	0% 0/10	0.06
	SRL ($n=10$)	30% 3/10	10% 1/10	30% 3/10	30% 3/10	

2.3. Western blots

The mean arterial tissue content of SRL was 0.32 ± 0.24 ng/mg arterial tissue at 90 days. PCNA and $p27^{kip1}$ expression for control and SRL stents at 3, 30 and 90 days are demonstrated in Fig. 4. Western blot analysis failed to detect increased levels of expression of MCP-1, CD45, IL-2, IL-6, and TNF-β above normal non-injured sections of the aorta.

3. Discussion

The present study documents the temporal vascular response for SRL-eluting stents to 180 days in the porcine coronary model. The SRL stent effectively suppresses neointimal formation for the first 30 days in comparison with bare metal stents. Late neointimal formation occurs

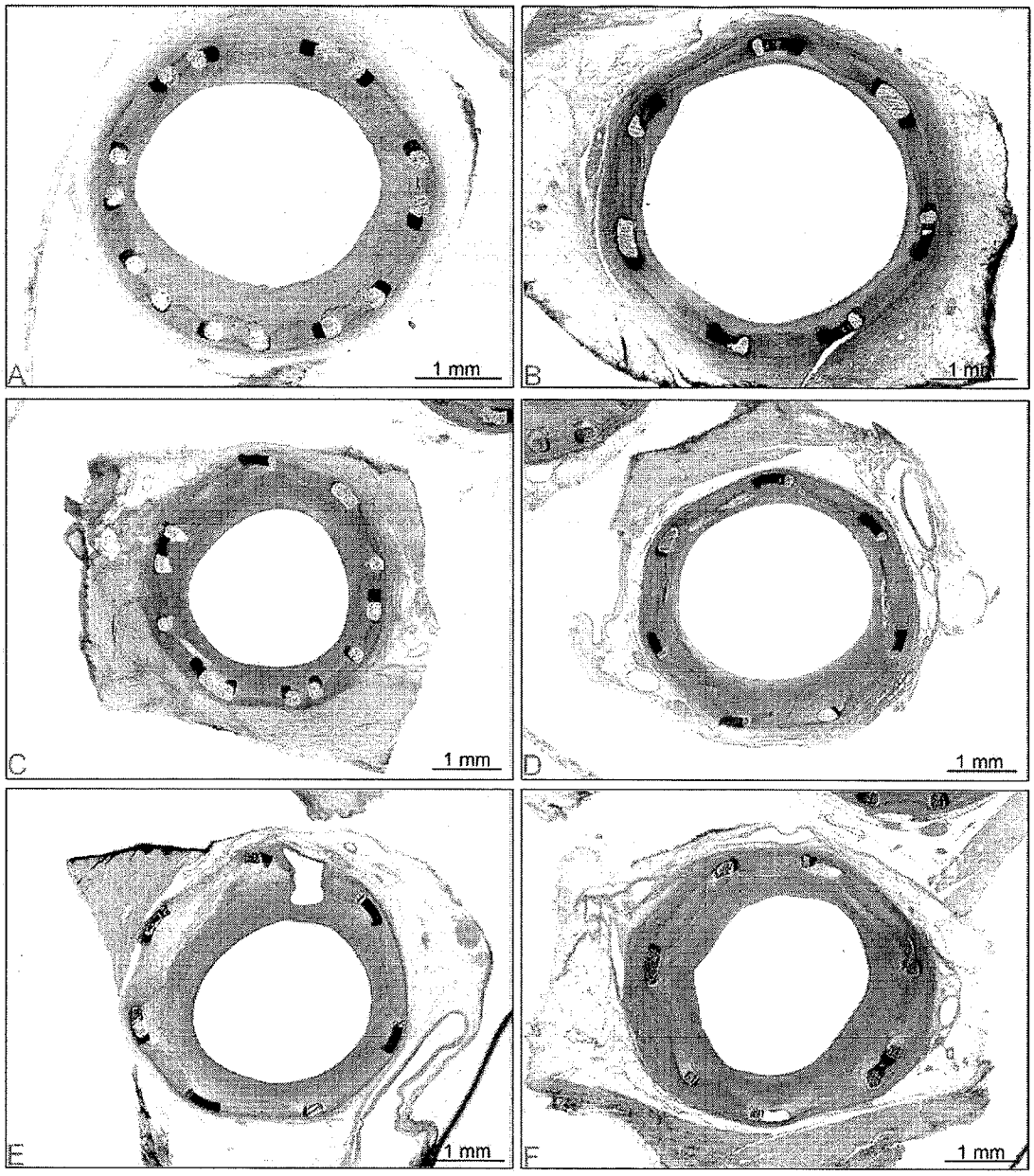


Fig. 2. Low power photomicrographs at 30, 90 and 180 days after placement of control and SRL eluting stents in porcine coronary arteries. At 30 days, neointimal area is greater with bare metal (A) as compared to the SRL stent (B). At 90 and 180 days, neointimal area and morphology are similar for bare metal (C, 90 days and E, 180 days) and SRL stents (D, 90 days and F, 180 days) (Metachromatic stain).

between 30 and 90 days for SRL stents at least in part due to inflammation and cellular proliferation. Angiographic and histological data documents a similar minimal degree of angiographic %diameter obstruction and mean neointimal area for the SRL and bare metal stents at 90 and 180 days. These observational data failed to identify medial necrosis, aneurysm formation, or excessive thrombosis for the SRL stents. Together, these data provide additional insights into the mechanism and efficacy of the SRL-eluting stent in normal porcine coronary arteries while raising questions regarding the potential durability of this drug eluting vascular prosthesis.

3.1. Experimental models of restenosis and drug-eluting stents

Previous studies have documented efficacy of SRL-eluting stents in two experimental models at 30 days [1,2]. In addition, these studies also supported evidence of polymer biocompatibility in two species at 60 days after

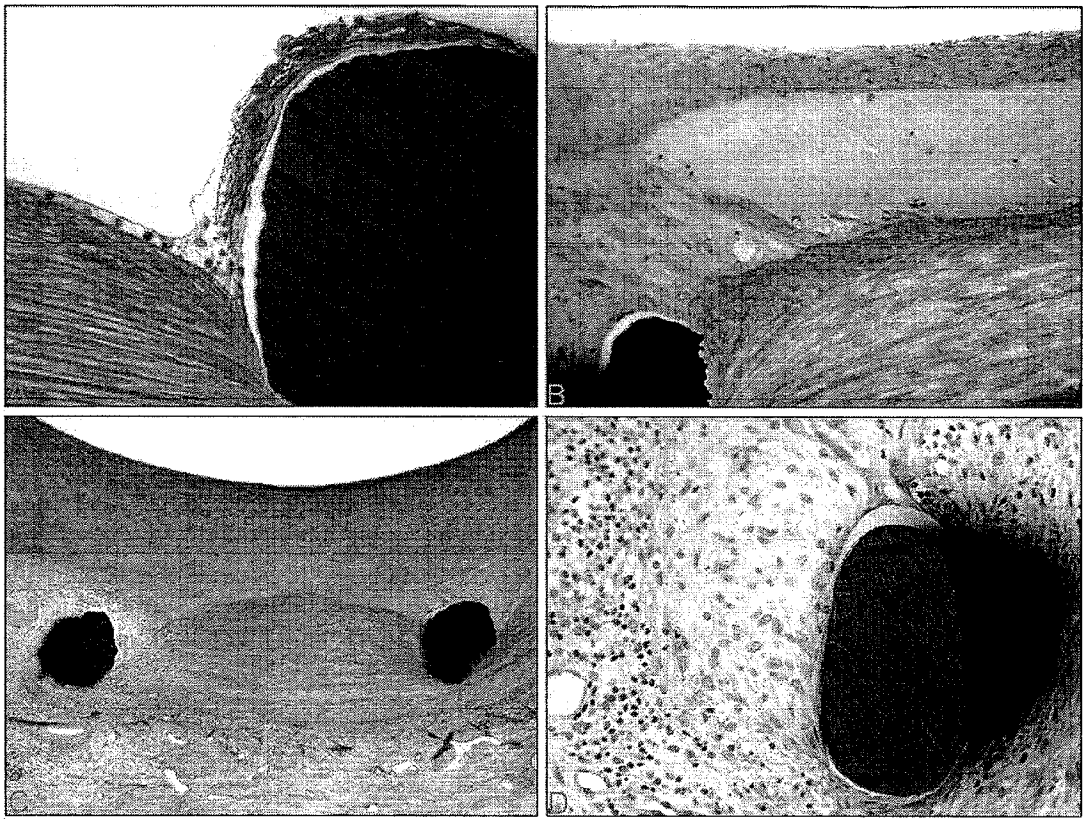


Fig. 3. High power photomicrographs at 3, 30, 90, and 180 days after placement of SRL stents in normal porcine coronary arteries. (A) At 3 days, a thin layer of thrombus is present over the stent strut. (B) At 30 days, neointima consists of SMC and amorphous material, presumably fibrin or "plasma-like" collection adjacent to the stent strut (black rectangle). (C) At 90 days, the neointima consists of SMC and matrix proteoglycans. The amorphous material observed at 30 days is no longer present. The media is intact except for a focal region of strut penetration associated with foreign body response. (D) At 180 days, the neointima demonstrates a localized lymphocytic reaction adjacent to a stent strut typical for the model (Metachromatic stain, original magnifications A=40×, B=21×, C=9×, D=33×).

experimental intracoronary stent placement [1]. An objective of the present study was to validate prior experimental observations and to characterize the chronic effects of the SRL-eluting stent on neointimal formation, regulatory proteins of the cell cycle and expression of inflammatory cytokines in a porcine coronary model.

In the porcine coronary model, studies employ oversized stent placement, typically 10% to 30% greater than the baseline vessel dimensions, to induce injury and neointimal formation. Over a decade has passed since Schwartz et al. [3] demonstrated the strong relationship between stent-induced vessel injury and neointimal formation at 28 days in the porcine coronary model. Subsequent studies by Kornowski et al. [4] and Welt and Rogers [5] documented the interactions of vessel wall injury and inflammation in contributing to neointimal formation at 28 days. Unfortunately, only limited published data exists to characterize long-term response to stenting in this model. Should we expect the treatment effect with SRL-eluting stents to persist beyond 30 days in the porcine coronary model?

In the present study, the mean neointimal area was approximately 50% less for the SRL versus the control stents at 30 days. In contrast, by 90 days, the mean neointimal area was similar for the SRL stents ($\approx 3.00\ mm^2$), as compared with control stents resulting in similar percent area in-stent stenosis. Light microscopy and SEM (unpublished data on file) of the SRL stents documented complete coverage of the luminal surface with endothelial cells within 30 days. Our data document detection of SRL (0.32 ng/mg) in the arterial tissue at 90 days with evidence of increased levels of $p27^{kip1}$, a mediator of the antiproliferative effects for this compound, in the vessel wall [6]. The expression of PCNA, a marker of cell proliferation, was more abundant in the vessel wall after 30 days for SRL eluting stents in contrast to control stents. These data suggest that other cell cycle regulators could also participate in SRL-mediated in-vivo inhibition of SMC proliferation, the possibility of an insufficient arterial drug level at 90 days, or perhaps the presence of other potent sustained physiologic stimuli of SMC proliferation and neointimal formation not sufficiently affected by SRL-mediated inhibition of the cell cycle [7].

Histological data documents a progressive increase in injury and inflammation scores between 30 and 180 days for the SRL as compared with control stents. This observed

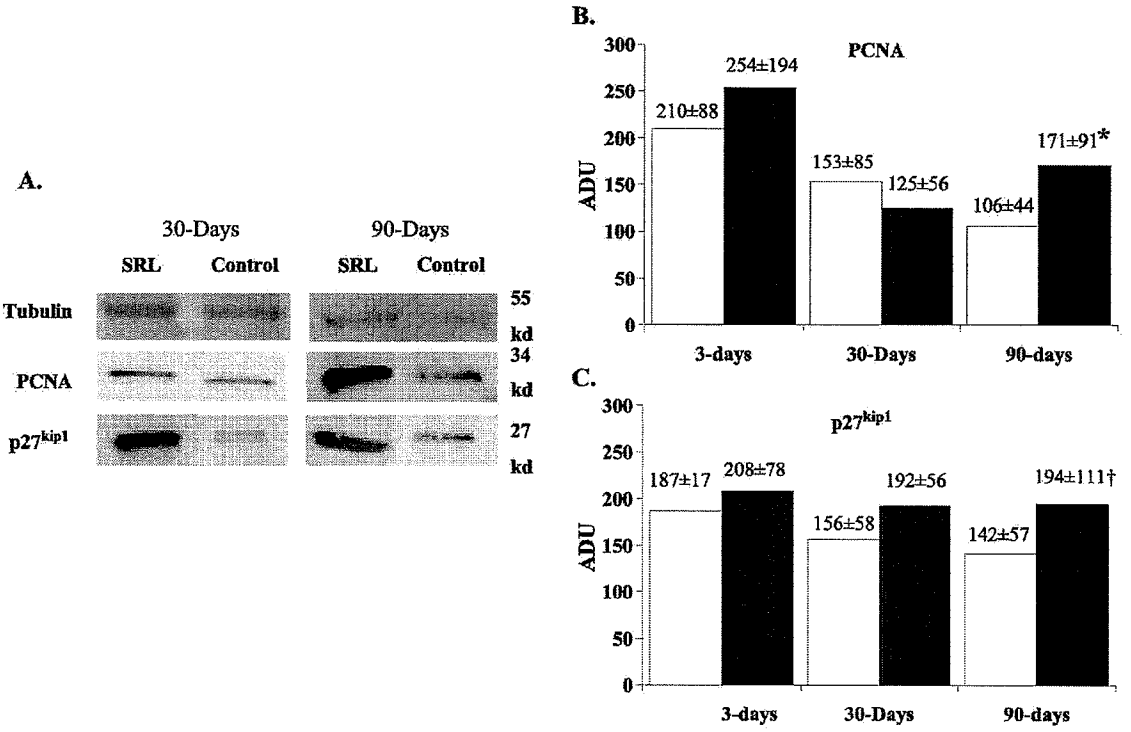


Fig. 4. (A) Representative Western blots at 30 and 90 days for arterial segments treated with bare metal control and SRL stents. Vascular segments treated with SRL stents demonstrated elevated PCNA levels at 90 days in comparison with bare metal control stents despite up-regulation of $p27^{kipl}$. (B and C) Bar graphs summarizing the densitometric analysis of Western blots demonstrate increased levels of $p27^{kipl}$ ($^{\dagger}p = 0.05$) but with significantly greater expression of PCNA at 90 days for SRL stents (dark bars) versus control stents (open bars) ($^{*}p = 0.003$). Data is expressed as mean ± S.E.M. of four separate experiments. ADU—Arbitrary Density Unit.

progression of injury and inflammation beyond 30 days likely represents a chronic vascular response to the drug or polymer. The character of the chronic inflammatory response, a predominantly lymphocytic reaction with giant cells, is consistent with a typical foreign body granulomatous response to a vascular prosthesis. The absence of eosinophils associated with a more severe diffuse inflammatory cell infiltration of the vessel wall, or increased levels of cytokines reduces the likelihood that the chronic inflammation observed in the present study is indicative of a hypersensitivity reaction to the drug or polymer.

Nonetheless, the vascular response to ongoing injury and inflammation induced by the stent with residual polymer may simply overwhelm the biological effects of the drug in this model and result in the late formation of neointima. Other physiologic factors such as vessel wall shear, apoptosis, matrix synthesis and degradation may also influence the long-term response to the SRL eluting stent in the porcine coronary model.

3.2. Comparison with clinical data for sirolimus-eluting stents

The safety and feasibility of the SRL-eluting stent were evaluated in a 45 patient phase I clinical trial and documented a stable in-stent MLD for the SRL-eluting stents after 2 years [8,9]. The RAVEL and SIRIUS randomized clinical trials have documented a significant reduction in clinical and angiographic restenosis at 12 months for SRL-eluting versus the BX Velocity stent in patients with focal de novo native coronary arterial lesions [10–13]. Recent data from the RAVEL trial have revealed a significantly lower frequency of target vessel revascularization at 3 years for SRL-eluting versus bare metal stents, despite four cases of target vessel revascularization between 1 and 3 years in the SRL group [13].

The clinical efficacy of SRL-eluting stents would not be expected based on the degree and duration of suppression of neointimal formation documented in normal porcine coronary arteries. The vastly different pharmacodynamics of SRL-eluting stents observed to date in human clinical trials versus preclinical models may be attributed to differences in species response to SRL, anatomic substrate and physiological stimulus for neointimal formation. We have previously reported and confirm in the present study a 50% reduction for SRL stents in comparison with control stents at 30 days in the porcine coronary model [1]. In the present study, the treatment effect for SRL stents was not observed beyond 30 days. In contrast, two randomized clinical trials have demonstrated a >90% inhibition of neointimal formation for SRL stents in comparison with bare metal stents as measured by volumetric IVUS after 6 months [7–9]. A precise

explanation for the discrepancy between preclinical and clinical results with SRL eluting stents remains elusive.

Wright et al. [14] have documented a two-fold difference in mitogen-stimulated peripheral blood mononuclear cells and mixed lymphocyte response for porcine versus human cells exposed to similar concentrations of SRL. A distinct species response to the antiproliferative and immunosuppressive effects of SRL may account in part for the disparity between 30-day porcine and 6-month human clinical data for this drug eluting stent. The vastly different anatomic and cellular substrate of atherosclerotic human versus normal porcine coronary arteries could also account for this dose response discrepancy. Zohlnhofer et al. [15] have demonstrated a higher prevalence of the FKBP-12 binding protein, the intracellular receptor for SRL, in intimal derived versus medial smooth muscle cells. Thus, perhaps a more abundant expression of the FKBP-12 receptor in atheroclerotic human coronary arteries in comparison with normal porcine coronary arteries enhances the efficacy of stent-based delivery of SRL in man. The differences in physiologic stimuli for neointimal formation are of obvious importance when comparing diseased human coronary arteries to normal porcine coronary arteries.

4. Conclusions

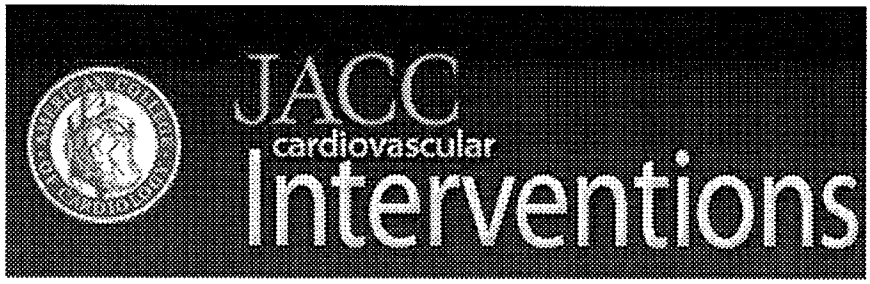
SRL-eluting stents favorably modulate neointimal formation for 30 days in the porcine coronary model. Long-term inhibition of neointimal hyperplasia was not sustained presumably due to delayed cellular proliferation, despite increased expression of the cyclin-dependent kinase $p27^{kip1}$. Our data highlight the necessity to improve our understanding of preclinical cardiovascular drug and device testing as well as to explore refinements of stent-based drug delivery. Randomized clinical trials with 3 to 5 years observation are necessary to document a sustained benefit for drug eluting stents.

Acknowledgements

The authors wish to acknowledge the expert technical assistance of Lori Gibson, DVM, Lisa Welch, Lynn Bailey, Julianne Siegel, in the completion of the experimental phases of this study. Anne Schwarzkof and Brooke Hallak conducted the quantitative analysis of coronary angiograms under the direction Edwin Alderman, MD in the CCAL, Stanford University Medical Center. This study was funded by the Medical Device Research Laboratory of the Providence Heart and Vascular Institute and a grant from Cordis.

References

[1] Suzuki T, Wilenski R, Bailey LR, et al. Stent-based sirolimus therapy reduces neointimal formation and in-stent restenosis in a porcine coronary model. Circulation 2001;104:1188–93.
[2] Klugherz BD, Llanos G, Lieuallen W, et al. Stent-based delivery of sirolimus for the prevention of restenosis. Coron Artery Dis 2002;13:183–8.
[3] Schwartz RS, Huber KC, Murphy JG, et al. Restenosis and the proportional neointimal response to coronary artery injury: results in a porcine model. J Am Coll Cardiol 1991;19:267–74.
[4] Kornowski R, Hong MK, Tio FO, Bramwell O, Wu H, Leon MB. In-stent restenosis: contributions of inflammatory responses and arterial injury to neointimal hyperplasia. J Am Coll Cardiol 1998;31:224–30.
[5] Welt FGP, Rogers C. Inflammation and restenosis in the Stent Era. Arterioscler Thromb Vasc Biol 2002;22:1769–76.
[6] Gallo R, Padurean A, Jayaraman T, et al. Inhibition of intimal thickening after balloon angioplasty in porcine coronary arteries by targeting regulators of the cell cycle. Circulation 1999;99:2164–70.
[7] Braun-Dullaeus RC, Mann MJ, Seay U, et al. Cell cycle protein expression in vascular smooth muscle cells in vitro and in vivo is regulated through phosphatidylinositol 3-kinase and mammalian target of rapamycin. Arterioscler Thromb Vasc Biol 2001;21:1152–8.
[8] Sousa JE, Costa MA, Abizaid AC, et al. Lack of neointimal proliferation after implantation of sirolimus-coated stents in human coronary arteries. Circulation 2001;103:192–5.
[9] Sousa JE, Abizaid A, Abizaid A, et al. Late (three-year) follow-up from the first-in-man (FIM) experience after implantation of sirolimus-eluting stents. Circulation 2002;106(Suppl. II):II-394 [Abstract].
[10] Morice MC, Serruys PW, Sousa JE, et al. A randomized comparison of a sirolimus-eluting stent with a standard stent for coronary revascularization. N Engl J Med 2002;346:1773–80.
[11] Serruys PW, Degertekin M, Tanabe K, et al. Intravascular ultrasound findings in the multicenter, randomized, double-blind RAVEL (RAndomized study with the sirolimus-eluting VElocity balloon-expandable stent in the treatment of patients with de novo native coronary artery Lesions) trial. Circulation 2002;106:798–803.
[12] Moses JW, Leon MB, Popma JJ, et al. Sirolimus-eluting stents versus standard stents in patients with stenosis in a native coronary artery. N Engl J Med 2003;349:1315–23.
[13] Morice MC, Serruys P, Constantini C, et al. Three-year follow-up of the RAVEL study: A randomized study with the sirolimus-eluting velocity balloon-expandable stent in the treatment of patients with de novo native coronary artery lesions. J Am Coll Cardiol 2004;43:87A [Abstract].
[14] Wright DC, Deol HS, Tuch BE. A comparison of the sensitivity of pig and human peripheral blood mononuclear cells to the antiproliferative effects of traditional and newer immunosuppressive agents. Transpl Immunol 1999;7:141–7.
[15] Zohlnhofer D, Klein CA, Richter T, et al. Gene expression profiling of human stent induced neointima by cDNA array analysis of microscopic specimens retrieved by helix cutter atherectomy: detection of FK506-binding protein 12 upregulation. Circulation 2001;103:1396–402.



A Randomized, Controlled, Multicenter Trial to Evaluate the Safety and Efficacy of Zotarolimus- Versus Paclitaxel-Eluting Stents in De Novo Occlusive Lesions in Coronary Arteries: The ZoMaxx I Trial

Bernard Chevalier, Carlo Di Mario, Franz-Josef Neumann, Flavio Ribichini, Philip Urban, Jeffrey J. Popma, Peter J. Fitzgerald, Donald E. Cutlip, David O. Williams, John Ormiston, Eberhard Grube, Robert Whitbourn, Lewis B. Schwartz, for the ZoMaxx I Investigators

J. Am. Coll. Cardiol. Intv. 2008;1;524-532
doi:10.1016/j.jcin.2008.08.010

JACC: CARDIOVASCULAR INTERVENTIONS VOL. 1, NO. 5, 2008
© 2008 BY THE AMERICAN COLLEGE OF CARDIOLOGY FOUNDATION ISSN 1936-8798/08/$34.00
PUBLISHED BY ELSEVIER INC. DOI: 10.1016/j.jcin.2008.08.010

A Randomized, Controlled, Multicenter Trial to Evaluate the Safety and Efficacy of Zotarolimus- Versus Paclitaxel-Eluting Stents in De Novo Occlusive Lesions in Coronary Arteries

The ZoMaxx I Trial

Bernard Chevalier, MD,* Carlo Di Mario, MD,† Franz-Josef Neumann, MD,‡
Flavio Ribichini, MD,§ Philip Urban, MD,‖ Jeffrey J. Popma, MD,¶ Peter J. Fitzgerald, MD, PHD,#
Donald E. Cutlip, MD,** David O. Williams, MD,†† John Ormiston, MD,‡‡
Eberhard Grube, MD,§§ Robert Whitbourn, MD,‖‖ Lewis B. Schwartz, MD,¶¶
for the ZoMaxx I Investigators

Saint-Denis, France; London, England; Bad Krozingen and Siegburg, Germany; Novara, Italy; Geneva, Switzerland; Boston, Massachusetts; Palo Alto, California; Providence, Rhode Island; Auckland, New Zealand; Melbourne, Australia; and Abbott Park, Illinois

Objectives A novel zotarolimus-eluting coronary stent system (ZoMaxx, Abbott Laboratories, Abbott Park, Illinois) was compared with a paclitaxel-eluting coronary stent (Taxus Express2) in a randomized trial of percutaneous intervention for de novo coronary artery stenosis. The primary end point was defined as noninferiority of in-segment late lumen loss after 9 months.

Background The ZoMaxx stent system elutes 10 μg/mm zotarolimus using a phosphorylcholine polymer loaded onto a novel stainless steel stent platform containing a 0.0007-inch inner layer of tantalum.

Methods Twenty-nine investigative sites in Europe, Australia, and New Zealand enrolled 401 patients, 396 of whom received a study stent.

Results After 9 months, late lumen loss was significantly greater in the ZoMaxx group (in-stent 0.67 ± 0.57 mm vs. 0.45 ± 0.48 mm; $p < 0.001$; in-segment 0.43 ± 0.60 mm vs. 0.25 ± 0. 45 mm; $p = 0.003$), resulting in significantly higher rates of >50% angiographic restenosis (in-stent 12.9% vs. 5.7%; $p = 0.03$; in-segment 16.5% vs. 6.9%; $p = 0.007$). The upper bound of the 95% confidence interval on the difference in in-segment late lumen loss between the 2 treatment groups (0.27 mm) exceeded the 0.25 mm value pre-specified for noninferiority. There were no significant differences between ZoMaxx and Taxus-treated groups with respect to target lesion revascularization (8.0% vs. 4.1%; $p = 0.14$), major adverse cardiac events (12.6% vs. 9.6%; $p = 0.43$), or stent thrombosis (0.5% in both groups).

Conclusions After 9 months, the ZoMaxx stent showed less neointimal inhibition than the Taxus stent, as shown by higher in-stent late loss and restenosis by qualitative coronary angiography. (J Am Coll Cardiol Intv 2008;1:524–32) © 2008 by the American College of Cardiology Foundation

From the *Centre Cardiologique du Nord; Saint-Denis, France; †Royal Brompton Hospital; London, England; ‡Herz-Zentrum Bad Krozingen; Bad Krozingen, Germany; §Università del Piemonte Orientale; Novara, Italy; ‖La Tour Hospital; Geneva, Switzerland; ¶Brigham & Women's Hospital; Boston, Massachusetts; #Stanford University Medical Center; Palo Alto, California; **Harvard Clinical Research Institute; Boston, Massachusetts; ††Rhode Island Hospital; Providence, Rhode Island; ‡‡Auckland City Hospital; Auckland, New Zealand; §§Heart Center Siegburg; Siegburg, Germany; ‖‖St. Vincent's Hospital; Melbourne, Australia; and ¶¶Abbott Laboratories; Abbott Park, Illinois. Dr. Chevalier has received research support from and is a consultant for Abbott Laboratories; Dr. DiMario is on the Speaker's Bureau and has received honoraria and/or is a consultant for Abbott Laboratories and Boston Scientific; Dr. Urban has received research support from, is on the Speaker's Bureau, and/or is a consultant for Biosensors, Johnson & Johnson, Medtronic, and Terumo; Dr. Popma has received research support from, is on the Speaker's Bureau of, and/or is a consultant for Abbott Laboratories, Boston Scientific, eV3, Johnson & Johnson, Eli Lilly and Company, Medtronic, and Sanofi; Dr. Fitzgerald has received research support from and is a consultant for Abbott Laboratories; Dr. Williams is a consultant for Abbott Laboratories; Dr. Ormiston has received research support or honoraria from and/or is a consultant for Abbott Laboratories and Boston Scientific; Dr. Schwartz is a full-time employee of Abbott Laboratories. Presented at Transcatheter Cardiovascular Therapeutics Washington, DC, October 23, 2006.

Manuscript received June 30, 2008; revised manuscript received July 21, 2008, accepted August 20, 2008.

JACC: CARDIOVASCULAR INTERVENTIONS, VOL. 1, NO. 5, 2008
OCTOBER 2008:524–32

Drug-eluting stents (DES) are intravascular metal scaffolds that are coated with antiproliferative agents designed to treat critical occlusive lesions of the coronary arteries. Stents eluting either sirolimus, paclitaxel, everolimus, or zotarolimus have each been shown to effectively inhibit restenosis in large-scale clinical trials (1–4). First introduced in 2001, DES have been widely applied, with over 6 million patients treated worldwide (5).

See page 533

The clinical trial reported herein was designed to compare the novel ZoMaxx zotarolimus-eluting stent (Abbott Laboratories, Abbott Park, Illinois) with the Taxus Express2 paclitaxel-eluting stent (Boston Scientific Corporation, Natick, Massachusetts). The ZoMaxx stent uses the TriMaxx (Abbott Laboratories) stainless steel–tantalum stent platform to deliver zotarolimus 10 μg/mm via a well-characterized polymer system based on phosphorylcholine (PC) (6). The objective of this randomized, prospective, multicenter trial was to show the safety and efficacy of the ZoMaxx stent system as compared with the Taxus Express2 stent system for patients with single de novo lesions in native coronary arteries using clinical, angiographic, and intravascular ultrasonic methods.

Methods

Study design and end points. The ZoMaxx I trial was a randomized, prospective, multicenter clinical trial conducted in accordance with the International Conference on Harmonization guidelines–Good Clinical Practices, Declaration of Helsinki, International Organization for Standardization 14155-1, International Organization for Standardization 14155-2, and Ethics Committee requirements. All patients gave written informed consent for participation.

Patients were considered eligible for inclusion if they complained of stable or unstable angina and/or had objective evidence of myocardial ischemia with angiographically proven single >50% lesions of 10 to 30 mm in length in 2.5- to 3.5-mm native coronary arteries. The major clinical exclusion criteria were acute myocardial infarction within the past 72 h, impaired left ventricular function with ejection fraction <30%, or lesions located within the left main coronary artery or within 2.0 mm of their ostia.

Secondary end points included device success (achievement of a final residual in-stent diameter stenosis of <30% using the assigned device only), lesion success (<30% residual stenosis using any percutaneous method), procedure success (lesion success without the occurrence of major adverse cardiac events [MACE]), angiographic rates of binary restenosis (≥50% diameter stenosis) after 9 months, neointimal hyperplasia volume after 9 months as measured by intravascular ultrasound (IVUS), and the 9-month incidences of ischemia-driven target lesion revascularization (TLR), ischemia-driven target vessel revascularization (TVR), and MACE (composite end point of non–Q-wave myocardial infarction [MI], Q-wave MI, TVR, and cardiac death). The first 250 randomized subjects with IVUS-eligible lesions were required to have IVUS evaluation during the procedure and at 9-month follow-up. A Q-wave MI was defined as the development of new pathological Q-waves in 2 or more contiguous leads with post-procedure creatine kinase (CK) or CK-MB levels elevated above normal. Non–Q-wave MI was defined as elevation of post-procedure CK levels to >2.0 times normal with elevated CK-MB in the absence of new pathological Q waves (World Health Organization definition). Acute luminal gain was defined as the difference between the minimum lumen diameter (MLD) at the completion of the stenting procedure and at baseline. Data are expressed as the mean ± SD for continuous variables and as frequencies for categorical variables (SAS Statistical Analysis Software for Windows, version 8.2, SAS Institute Inc., Cary, North Carolina).

Abbreviations and Acronyms

CK = creatine kinase
DES = drug-eluting stents
IVUS = intravascular ultrasound
MACE = major adverse cardiovascular events
MLD = minimum lumen diameter
PC = phosphorylcholine
TLR = target lesion revascularization
TVR = target vessel revascularization

Stent system. The ZoMaxx stent was designed to address the need for thin strut width and low profile, while maintaining radial strength and adequate visibility on fluoroscopy. The stent metal is a trilayer composite having 2 outer layers of 316L stainless steel and an inner layer of tantalum (Fig. 1) (7). The high atomic number of the 18-μm inner tantalum layer affords optimal radiopacity of the thin stent struts. The result is a DES with a strut thickness of only 0.0029 inches (74 μm), an important metric for minimizing arterial injury and restenosis (8–10).

The ZoMaxx stent elutes 10 μg/mm zotarolimus via a biocompatible PC polymer. Zotarolimus (Fig. 2) was specifically developed for use on intravascular stents and, like sirolimus, reversibly binds to FKBP-12, the cytosolic receptor of FK506 (11,12). Using this mechanism, zotarolimus inhibits the activation and proliferation of a variety mammalian cells at very low concentrations. Its potency for inhibition of human lymphocytes in vitro has been shown, as well as the reduction of inflammation in animal models of arthritis and encephalomyelitis (13). In cultured vascular cells, zotarolimus inhibits proliferation of canine and human smooth muscle and endothelial cells with IC_{50} in the low nanomolar range (11,12,14). It has minimal effects on cellular migration in vitro, theoretically allowing re-

JACC: CARDIOVASCULAR INTERVENTIONS, VOL. 1, NO. 5, 2008
OCTOBER 2008:524–32

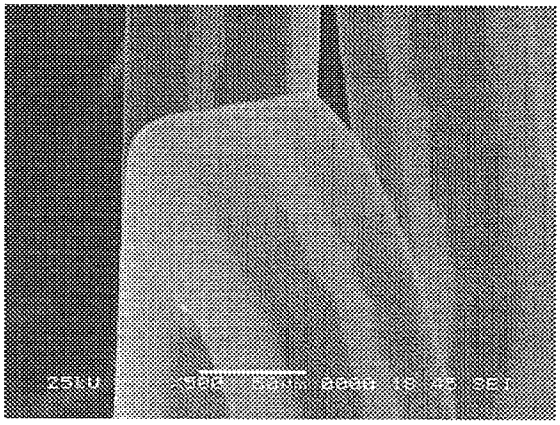


Figure 1. The ZoMaxx Stent Platform Consisting of a Trilayer of Stainless Steel, Tantalum, and Stainless Steel

endothelialization to proceed normally in the presence of the drug.

The phosphorylcholine polymer drug carrier on the ZoMaxx stent, known simply as PC-1036 or PC, is composed of the polymers 2-methacryloyloxyethyl phosphorylcholine (MPC), lauryl methacrylate (LMA), hydroxypropyl methacrylate (HPMA), and trimethoxysilylpropyl methacrylate (TSMA) in the molar ratios of MPC_{23}, LMA_{47}, $HPMA_{25}$, and $TSMA_5$ (6,15). It has been experimentally shown to have several properties improving blood–biomaterial compatibility, including minimal induction of fibrinogen absorption, platelet activation, platelet and erythrocyte adherence (15–17), inflammation, and neointimal hyperplasia (6,17–20). Phosphorylcholine has an extensive record of widespread clinical use and safety (6,21–23). It has also recently been shown to have considerable potential as a vehicle for drug elution, especially using highly lipophilic agents. It is formulated on the ZoMaxx stent to provide a measured rate of elution so that, in experimental animals, about 60% of the total zotarolimus dose is released during the first week, an additional 20% during the second week, and the remaining 20% over the next 2 weeks (24).

Treatment protocol. After patient eligibility was established and written consent obtained, the lesions were approached according to standard institutional interventional techniques. Patients received oral antiplatelet therapy with aspirin (100 mg/day) and clopidogrel (75 mg/day) starting before the procedure and continuing for 6 months. After percutaneous access, heparin was administered to maintain an activated clotting time ≥250 s (or ≥200 s if glycoprotein IIb/IIIa antagonism was used). Diagnostic coronary angiography was performed in matched orthogonal views after nitroglycerin coronary injection (50 to 200 μg). After a 0.014-inch wire crossing of the target lesion, randomization (1:1 ZoMaxx vs. Taxus) was performed via an interactive telephone system.

Balloon pre-dilation of the target lesion was mandatory and was performed according to standard techniques ensuring that the length of the balloon was no greater than the intended stent length. Direct stenting was prohibited in this study. ZoMaxx stents were available in diameters of 2.5, 3.0, and 3.5 mm with lengths of 8, 18, 23, 28 mm (2.5-mm diameter only), and 33 mm (3.0- and 3.5-mm diameters only). Taxus stents were available in diameters of 2.5, 3.0, and 3.5 mm with lengths of 8, 12, 16, 20, 24, 28 mm (3.0- and 3.5-mm diameters only) and 32 mm (3.0- and 3.5-mm diameters only). Only 1 study stent was to be used per patient; however, additional stents could be implanted at the operator's discretion in the event of edge dissection or incomplete coverage.

Intravascular ultrasound images were acquired by motorized pullback at a constant speed of 0.5 mm/s. Baseline, post-procedure, and 9-month follow-up coronary cineangiographic images (Medis CMS, Leiden, the Netherlands) and IVUS tapes (TapeMeasure, Indec Systems, Inc., Mountain View, California) were analyzed using independent core laboratories (Brigham and Women's Hospital, Boston, Massachusetts, and Stanford University Medical Center, Palo Alto, California, respectively). Imaging studies performed within 284 days (9 months + 2 weeks) were included in the analysis.

Monitoring and statistical analysis. The study was monitored by independent contract research organizations (Hesperion AG, Allschwil, Switzerland, and Clinimetrics Research Associates, Inc., San Jose, California) and data were coordinated and analyzed by the Harvard Clinical Research Institute (Boston, Massachusetts). All MACE were re-

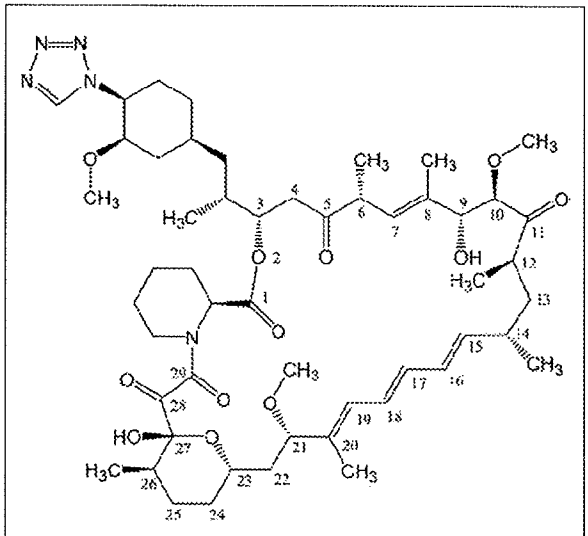


Figure 2. The Molecular Structure of Zotarolimus